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I. Mirex and Kepone

Battelle Columbus Labs, OH

Prepared for

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Reviews of the Environmental Effects of Pollutants:

I. Mirex and Kepone

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The objective of this research program was to assemble and analyze the publicly-available information on the environmental effects of the two organochlorine pesticides, mirex (C10C112) and its ketone analog, Kepone (C10C112). The data base on these compounds is limited, especially with respect to Kepone, on which almost no environmental research was conducted prior to the Life Science's Products Company incident. Both of these compounds are non-volatile, have very low water solubilities, are chemically and thermally very stable, and are quite resistant to biodegradation, thus, existing environmental residues can be expected to persist for years. Neither mirex nor Kepone appreciably affects microorganisms or plants in usual environmental concentrations. Mirex does not appear to present a serious acute toxicity problem to non-target terrestrial invertebrates as a result of fire ant control efforts. Both mirex and Kepone produce adverse reproductive effects in numerous species. Mirex has been shown to induce liver tumors in mice and rats and is judged to have potent , ...

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REVIEWS OF THE ENVIRONMENTAL EFFECTS OF POLLUTANTS:
I. MIREX AND KEPONE

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FOREWORD

A vast amount of published material is accumulating as numerous research investigations are conducted to develop a data base on the adverse effects of environmental pollution. As this information is amassed, it becomes continually more critical to focus on pertinent, well-designed studies. Research data must be summarized and interpreted in order to adequately evaluate the potential hazards of these substances to ecosystems and ultimately to public health.

The series of documents entitled "Reviews of the Enviornmental Effects of Pollutants" (REEPs) represents an extensive compilation of relevant research and forms an up-to-date compendium of the environmental effect data on selected pollutants.

The Review of the Environmental Effects of Mirex and Kepone includes information on the chemical and physical properties of both compounds; pertinent analytical techniques; transport processes to the environment and subsequent distribution and deposition; impact on microorganisms, plants, and wildlife; toxicologic data in experimental animals including metabolism, toxicity, mutagenicity, teratogenicity and carcinogenicity; and an assessment of their health effects in man. The volume of factual information presented on Mirex and Kepone in this document is summarized and interpreted in the final chapter "Environmental Assessment", which presents an overall evaluation of the potential hazard resulting from present concentrations of mirex and kepone in the environment. This final chapter represents a major contribution by Dr. Earl G. Alley from Mississippi State University.

The REEPS are intended to serve various technical and administrative personnel within the Agency in the decision-making processes; i.e. in the development of criteria documents and environmental standards, and for other regulatory actions.*

* The breadth of these documents makes them a useful resource for public health personnel, environmental specialists, and control officers. Upon request these documents will be made available to any interested individuals or firms, both in and out of the Government. Depending on the supply, you can obtain the document directly by writing to:

U.S. EPA
Environmental Criteria and Assessment Office
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PREFACE

Mirex and Kepone are two members of the family of organochlorine pesticides whose environmental effects have, until recently, been relatively ignored when compared with such compounds as DDT, chlordane, aldrin, and dieldrin. However, mirex and Kepone are two of the most stable and persistent members of this family. Recent events have made it increasingly apparent that the environmental effects of these compounds needed to be appraised.

Both mirex and Kepone are relatively recently used compounds. They have been manufactured commercially for only about 25 years; each was basically a one company product, and each had only limited, specialized pesticidal use. However, their environmental impacts have not been inconsequential. Millions of acres in the southeastern United States have been treated with mirex for control of the imported fire ant, and the extent of inadvertent contamination with Kepone from the Life Sciences Products Company is well known.

Unfortunately, there have been few investigations of their health and environmental effects, and much of the data on these substances are proprietary in nature. The present document has attempted to consider all of the open literature and some of the internal reports made available to the authors. However, because of the lack of extensive research, the anticipation and assessment of all potential environmental effects is not possible. The use of these pesticides has been banned; therefore, the present environmental burden should decrease as a result of their biodegradation, even through they are relatively stable and will be present in the environment for a long time.

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ABSTRACT

The objective of this research program was to assemble and analyze the publicly-available information on the environmental effects of the two organochlorine pesticides, mirex ($C_{10}Cl_{12}$) and its ketone analog, Kepone ($C_{10}Cl_{12}$). The data base on these compounds is limited, especially with respect to Kepone, on which almost no environmental research was conducted prior to the Life Science's Products Company incident. Both of these compounds are non-volatile, have very low water solubilities, are chemically and thermally very stable, and are quite resistant to biodegradation, thus, existing environmental residues can be expected to persist for years.

Neither mirex nor Kepone appreciably affects microorganisms or plants in usual environmental concentrations. Mirex does not appear to present a serious acute toxicity problem to non-target terrestrial invertebrates as a result of fire ant control efforts. Both mirex and Kepone produce adverse reproductive effects in numerous species. Mirex has been shown to induce liver tumors in mice and rats and is judged to have potential carcinogenicity, and carcinogenic properties of Kepone have been documented in mice and rats.

Both mirex and Kepone are lipid-soluble and accumulate in fatty tissues, from which the rate of excretion is slow. Mirex has been detected in adipose tissues of humans and in beef fat from cattle in areas aerially treated for fire ant control, but there are few definitive data on its effects on humans, and exposures appear to have been minimal. Exposures of humans to Kepone in roach and ant traps appears also to have been minimal. Excessive exposures did result from the inadequate control of Kepone during its manufacture at LSPC. No practical scheme for the removal or neutralization of the Kepone contained in James River sediments downstream of this plant have yet been devised, and this environmental inventory is likely to pose a problem for many years.

This report was submitted in fulfillment of Contract No. 68-03-2608 by Battelle's Columbus Laboratories under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period from April 1976 to August 31, 1978, and work was completed as of September 30, 1978.

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1.0 EXECUTIVE SUMMARY

Mirex and Kepone^{R}[*] are two of the more recent additions to the large family of organochlorine compounds used to combat insect pests. They are chemically quite similar. Mirex is an extremely stable and persistent compound containing only carbon and chlorine atoms, with the formula $C_{10}Cl_{12}$. Kepone has an analogous ketone structure, with two of the chlorine atoms replaced with an oxygen atom, $C_{10}Cl_{10}O$. Mirex has been widely used in the southeastern United States for control of the imported fire ant, via aerial broadcasting of a mirex - containing bait. In these areas mirex has been detected in a number of non-target organisms, including man. Recent improvements in bait formulation appear to make effective control possible at application rates of below 2.5 g/ha (1 g/ac) of active ingredient.

The estimated quantity of mirex applied for fire ant control between 1959 and 1977 is approximately 275,000 kg (600,000 lb). Recently promulgated EPA regulations permit aerial application only through December 31, 1977. The future use of mirex for control of the imported fire ant in the U.S. is uncertain.

Unlike mirex, Kepone has not been used widely in the U.S. Its principal domestic use is in ant and roach traps around homes and buildings. Most Kepone produced in the U.S. was exported to other countries for use against the banana root borer, and for conversion into other insecticides. Accordingly, the environmental effects of Kepone were little studied prior to the discovery of gross discharges of Kepone from the Life Sciences Products Company plant at Hopewell, Virginia, where it was manufactured. Severe contamination of the James River and its biota resulted, for which a solution has not yet been found.

After the discovery of the gross Kepone contamination from the Life Sciences Products Company, the plant was shut down and manufacture of Kepone terminated in July, 1975. Kepone is no longer being manufactured in the United States. Total production of Kepone was approximately 1,600,000 kg (3,500,000 lb) of which 90 to 95 percent appears to have been exported.

Kepone is a registered trademark of Allied Chemical Corporation.

1.1 TECHNOLOGY OF MIREX AND KEPONE

Both mirex and Kepone are produced from hexachlorocyclopentadiene. Both are moderately soluble in organic solvents but almost insoluble in water. Both compounds are highly resistant to chemical, thermal and biochemical degradation. Mirex is particularly stable in the environment, with a half-life estimated to be in excess of 10 years. Kepone is one of the principal biodegradation products of mirex. It's half-life is presumed to be nearly as long as that of mirex, so that Kepone residues in the environment will also be long-lasting.

Both mirex and Kepone residues in the environment are analyzed by gas chromatography (GC) using electron capture detectors. Polychlorinated biphenyls (PCB's), ubiquitous in the environment, are an interference in GC analysis of mirex, and can be misintrepreted as mirex unless eliminated by suitable pretreatment of the sample. Additionally, a chemically identical compound was also sold as Dechlorane®* for use as a flame retardant in plastics and polymers. This product was sold in quantities three times as large as those of mirex, and some of this may have been released into the biosphere.

Mirex was first used against the imported fire ant in 1961. Through 1975, about 400,000 kg (880,000 lb) of mirex were sold for agricultural use. On the basis of USDA records of mirex application, approximately 190,000 kg were used in the southeastern United States in the Federal-state program for control of the imported fire ant. Several thousand kilograms of mirex were also applied through independent state programs. Total U.S. use for fire ants is estimated to have been about 250,000 kg. Much of the other 150,000 kg was evidently exported. Considerable quantities were used in Brazil against the fire ant.

The U.S. Environmental Protection Agency, after a lengthy hearing on mirex, placed the use of mirex for fire ant control on a phase-down schedule, under which aerial application was to terminate December 31, 1977. Under this schedule, approximately another 25,000 kg of mirex were aerially applied in 1976 and 1977.

Kepone had essentially no agricultural uses in the U.S. Small quantities were used for control of the tobacco wireworm, and Kepone was registered for use against the banana root borer in Puerto Rico. Almost all (90 to 99 percent) of the 1,600,000 kg manufactured between 1959 and 1975 appears to have been exported.

The principal domestic use for Kepone was in ant and roach traps, around homes and businesses. Data on the quantities so used are not available, but Kepone concentration in the bait was only 0.125 percent, and the tonnages are estimated to have been relatively small.

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*Dechlorane is a registered trademark of Hooker Chemicals and Plastics Corporation.

1.2 EFFECTS ON MICROORGANISMS

Almost no data have been reported on the effects of mirex and Kepone on microorganisms. The few data which are available indicate very few effects at concentrations levels likely to be encountered an a result of normal usage of either pesticide. Sewage sludge microorganisms appear to be capable of metabolizing mirex under anaerobic conditions, but the process is very slow. Beneficial bacteria were reported killed in sewage treatment facilities at Hopewell, Virginia, upon the addition of large quantities of Kepone wastes.

1.3 EFFECTS ON PLANTS

Very few data are available on the effects of mirex and Kepone on plants. The growth of several clovers and grasses is inhibited by mirex in soil at concentrations of 0.15 to 0.70 ppm. Mirex accumulation in crop plant seedlings was shown to be directly related to soil concentration in the 0.3 to 3.5 ppm range; however, no data were presented on mirex translocation to edible plant parts. Mirex soil concentrations from aerial application of fire ant control bait are much lower than this, seldom exceeding a few ppb.

Plant root preparations known to metabolize other chlorinated hydrocarbons failed to yield any metabolite when incubated with mirex.

Essentially no data are available on the effects of Kepone on plants, and no data were found on the metabolism of Kepone by plants.

1.4 EFFECTS ON BIOTA

Mirex and Kepone have some differences in their effects on aquatic and terrestrial biota, but have a great many similar effects which is not surprising in view of their structural and chemical similarity.

Mirex, in realistic environmental concentrations, does not appear to be directly toxic to marine algae or freshwater plankton. Its extremely low solubility, 1 ppb or less, may contribute to this. However, bioaccumulation by algae is possible, and represents a possible route of entry into the food chain.

The effects of mirex on invertebrate species, especially marine crustaceans, vary from irritability to loss of equilibrium to death. Juvenile pink and brown shrimp and juvenile blue crabs are among the species most susceptible to mirex. Mirex is accumulated by fish from water and from food-chain organisms; it is concentrated mainly in the fatty tissue and visceral organs.

Mirex does not appear to present a serious acute toxicity problem to nontarget terrestrial invertebrates as a result of fire ant control efforts.

In comparison to mammals and fish, birds are not extremely sensitive to mirex in either acute or chronic exposures. However, reproductive difficulties such as decreased egg production, decreased survival of chicks, reduced hatchability, and eggshell abnormalities have been observed in several bird species. Wildlife sampling surveys in or near treated areas have shown that birds, as a group, contain relatively high levels of mirex.

Mirex does not have a high acute oral toxicity for those mammalian species which have been tested; many other organochlorine and organophosphate pesticides have much lower LD_{50}'s. The most commonly observed subacute toxic effects of mirex in mammals are weight loss, increased liver weight, and reproductive difficulties.

However, mirex has the highest chronicity factor observed in any pesticide examined to date. This factor compares the single dose LD_{50} with the 90-dose LD_{50}, and is an indication of the cumulative effects of substances without reference to their absolute toxicities. This fact, coupled with the slow excretion and lack of metabolism of mirex indicates that it is highly cumulative in effect.

Mirex (and Kepone) produce similar adverse reproductive effects in numerous species tested, including reductions in fertility and litter size, decreases in birthweight and survival of young, visceral anomalies, and elevated pesticide levels in offspring.

Mirex produces liver tumors in mice and rats and is judged to have potential carcinogenicity.

Kepone also has a low solubility, about 1 to 2 ppm in natural waters. Algal species accumulate Kepone from water and may pass the pesticide along the food chain to man. Kepone is acutely and chronically toxic to estuarine invertebrate species, and like mirex, may cause loss of equilibrium. Bioconcentration factors differ among invertebrate species because of differences in depuration rates; these are relatively rapid in oysters, but relatively slow in shrimp.

Kepone is bioaccumulated and persistent in fish. Symptoms of Kepone exposure may range from diminished activity and emaciation to abnormal development and mortality.

Data on effects of Kepone on nontarget terrestrial invertebrates are scant, but indicate it is not acutely toxic. Like mirex, Kepone has a low order of acute toxicity to birds, although in subacute dosages it is considerably more toxic than mirex. Tremors, pathologic changes in the liver, and reproductive difficulties similar to an "estrogenic effect" are seen in both sexes at dietary levels of 200 ppm of Kepone.

Kepone, both in oral and dermal administrations, is more acutely toxic than mirex in mammalian species. The most characteristic symptom of acute or subacute Kepone intoxication in mammals is severe and persistent tremor. Enlargement and congestion of the liver, weight loss, increased oxygen consumption, and urinary excretion of protein and sugar were reported in chronic toxicity studies on rodents and dogs.

Like mirex, excretion of body burdens of Kepone is slow. Similar to mirex, no metabolites of Kepone have been detected in Kepone-fed mice. As with mirex, the indications are that the biological stability of Kepone in the body is great. Excretion of the body burden of Kepone is slow, and no metabolites have been detected.

The reproductive effects of Kepone are similar to those previously described for mirex. In addition, oncogenic and carcinogenic properties of Kepone have been documented in rats and mice.

1.5 EFFECTS ON HUMANS

Human exposure to mirex and/or Kepone has been documented through analytical confirmation of residues of both pesticides in human tissues and man's food chain and, in the case of Kepone, by epidemiologic and medical surveillance of workers engaged in the manufacture of Kepone, and a systematic survey of a community where Kepone was manufactured.

Potential sources of exposure of humans to mirex are believed to be related to mirex bait application in fire ant control programs. There are few definitive data concerning the effects of mirex on humans. Neither acute nor chronic exposures appear to have been recorded, in either manufacture or use, and no clinical data were identified during this study.

Because mirex is a very stable insecticide capable of being stored in fat, the U.S. Department of Agriculture conducted a survey of the mirex content of beef fat from cattle which had grazed on treated pastures, in comparison to cattle from outside the treated areas. Residues were found in 88 percent of the beef fat samples from cows on treated pasture, with an average mirex content of 0.026 ppm, however, only one sample exceeded the established tolerance level of 0.1 ppm. No residues were found in organs, nor in the fat of cattle from outside the treated areas.

In another survey on the mirex content of milk, none of 60 samples from treated areas had detectable concentrations of mirex, at a detection limit of 0.3 ppb.

Mirex residues have been found in adipose tissue of humans living in treated areas. Approximately 19 percent of 284 samples analyzed had quantifiable levels of mirex, ranging from a trace to 1.2 ppm. Generally, the highest percentage of positive results correlated with the areas having a history of heaviest mirex usage.

Potential sources of exposure of humans to Kepone from its pesticidal use against ants and roaches appear to be minimal. No pesticidal uses involved dispersion on a large scale, as did mirex; the principal Kepone use was in baited insect traps, used domestically.

The major source of human exposure to Kepone resulted from its manufacture at the Life Sciences Products Company at Hopewell, Virginia. A large majority of the past and present employees at this plant exhibited symptoms of exposure to Kepone. Between the commencement of operations in March, 1974 and their termination in July, 1975, 76 (57 percent) of the workers contracted an illness compatible with Kepone toxicity. The principal manifestations of the poisoning were nervousness, tremor, weight loss, opsoclonus (erratic or jumpy eye oscillations) and pleuritic chest pains. These symptoms closely resembled those seen in animals suffering from Kepone intoxication.

Recent studies by physicians involved in treatment of these affected Kepone workers suggest that oral treatment with cholestryamine resin may be efficacious by facilitating Kepone excretion.

A systematic community survey, conducted to establish the geographic limits of the Kepone exposure area in Hopewell, showed that 40 of 216 blood samples contained from 5 to 50 ppb Kepone. Thirty-four of these 40 were from a residential area within 400 meters of the LSPC plant; none of the persons reported ever having worked with Kepone.

As a result of uncontrolled and unauthorized discharges of Kepone to the James River from the LSPC plant, the river sediments have been contaminated over a wide area to such an extent that "action levels" have been established by EPA for shellfish, finfish, and crabs.

Kepone has been detected in mother's milk gathered in the southeastern United States (9 of 298 samples), but at such low levels (<1 ppb to 6 ppb) that the hazards posed, if any, are uncertain.

1.6 ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

The principal route of entry of mirex into the environment has been the aerial application during the past 15 years of perhaps as much as 275,000 kg (600,000 lb) of mirex-dosed bait to millions of hectares in the southeastern U.S. for fire ant control. The corncob baits used contained variously 0.30, 0.15, or, most recently, 0.10 percent of mirex. Typical rates of application of mirex were extremely low, 4.2 g/ha (1.7 g/ac). At this rate, the calculated concentration of mirex in the top 7.5 cm (3 in) of soil would be 5 ppb or less, a remarkably low environmental concentration for an agricultural pesticide.

There is considerable uncertainty about the extent of dispersion to the environment of the approximately 1,125,000 kg (2,500,000 lb) of chemically identical compound sold for non agricultural use under the name Dechlorane. It's principal use was in plastics and polymers, to impart flame retardant properties. There is essentially no information on the ultimate fate of this far larger quantity of $C_{10}Cl_{12}$. If the plastic products containing the Dechlorane were disposed of in landfills instead of to an incinerator, the Dechlorane should be fairly well immobilized, and a source of only localized pollution. Additional investigation is needed to assess the environmental impacts of this use.

A second identified source of environmental dispersion of mirex results from losses during manufacture and handling of mirex/Dechlorane. Two "hot spots" have been discovered in Lake Ontario; one is presumed to have resulted from losses from the plant where mirex/Dechlorane was manufactured, and the other from a spill of Dechlorane fire retardant which occurred about 15 years ago. Surveys of Lake Ontario suggest that lake sediments may contain several hundred kilograms. These data suggest also that biodegradation is proceeding very slowly. Confirmation of the slow biodegradation of mirex is supplied by the detection of substantial residues in soils 5 and 12 years after mirex was deposited.

The measured solubility of mirex in water is 1 ppb or below, and mirex in natural waters in areas where mirex has been used is frequently undetectable at a detection level of 0.01 ppb. Dissolution of mirex in water does not appear to be a significant mode of transport. However, mirex accumulation has been noted in nontarget aquatic organisms. Concentrations resulting from its pesticide use have been below levels deemed harmful to man, but contamination levels in some species of Lake Ontario fish have resulted in a ban on their consumption.

There appear to be no data on concentrations of mirex in air, either in the vicinity of manufacturing sites or in areas where mirex has been applied. The low vapor pressure and the method of application of the mirex in a bait formulation suggests that ambient atmospheric concentrations are likely to be at the limit of detection or below.

The principal route of entry of Kepone into the enviroment was gross uncontrolled discharges from the plant which manufactured Kepone for the last year and one-half before its manufacture in the U.S. was terminated. Kepone had very minor agricultural uses, and its principal domestic use, in bait traps for ants and cockroaches, was not only a small tonnage use, but also did not significantly disperse it to the biosphere. Thus, prior to the Life Sciences Products Company incident, essentially no data existed on environmental concentrations of Kepone, and almost all of the environmental distribution data since acquired relate specifically to this incident.

Contamination of James River sediments from Hopewell, Virginia, nearly to Newport News has been demonstrated. Unacceptable concentrations have been detected in both invertebrates and fish, and upper limits have been established for oysters, clams, mussels, crabs, and finfish for human consumption. It has been estimated that thousands of kilograms may be trapped in James River sediments. No practical scheme for its removal or neutralization has yet been devised. Since Kepone, like mirex, is highly resistant to biodegradation, this inventory will pose a problem for many years.

Concentrations of suspended particulate Kepone in the atmosphere were variable and high near the LSPC plant during the period that Kepone was being manufactured. Except for this special case, Kepone would be expected to be undetectable in the atmosphere. In its normal use in bait traps for ants and roaches, the low vapor pressure of Kepone would preclude any significant release.

2.0 TECHNOLOGY OF MIREX AND KEPONE

2.1 SUMMARY

Mirex ($C_{10}Cl_{12}$) and Kepone^R ($C_{10}Cl_{10}O$) are related chemically, Kepone being the ketone analog of mirex. Accordingly, the compounds have many similarities. Both are produced from hexachlorocyclopentadiene; both are moderately soluble in organic solvents, and both have extremely low solubilities in water. Solubility of mirex in water is approximately 1 ppb (0.001 ppm); that of Kepone is higher, but still is low, only 1 to 2 ppm.

Mirex, consistent with its fully chlorinated structure, is one of the most stable organochlorine pesticides, and is highly resistant to chemical, thermal, and biochemical degradation. Thermal decomposition begins to occur at 550 to 600 C, and is rapid at 700 C. At these temperatures, degradation products include hexachlorobenzene and hexachlorocyclopentadiene. Mirex is resistant to photolysis in hydrocarbon solvents, but less so in aliphatic amines. Current research indicates that by using amines it may be possible to prepare mirex baits which decompose in 4 to 6 weeks. Mirex has a long half-life in the environment. Large fractional residues have been detected at two locations, 5 to 12 years after the initial application. Kepone was identified as one of the degradation products.

Kepone, with its carbonyl bond and its less fully chlorinated structure, is slightly less resistant than mirex to chemical and thermal degradation. Thermal decomposition begins to occur at about 350 C, and is rapid at 450 to 500 C. In addition to the hexachlorobenzene and hexachlorocyclopentadiene degradation products observed with mirex, a third product, possibly octachloroindene (C_9Cl_8), has been observed. When Kepone is exposed to 900 C for approximately 1 second, decomposition is essentially complete.

The usual method of analysis for both compounds at the extremely low concentration levels found in environmental samples is gas chromatography, using electron capture detectors. Interferences from polychlorinated biphenyls now ubiquitous in the environment have to be guarded against in the analysis. Since there are over 200 PCB isomers, several of which can masquerade as mirex, results reported in the literature may be suspect for this reason. Additionally, a chemical compound identical to mirex was sold for use as a flame retardant for polymeric and plastic materials under the name Dechlorane, in quantities three times as large as those of mirex. Some of the reported findings of mirex in the environment undoubtedly derived from this Dechlorane source.

Mirex has been used since 1962 in the southeastern states for control of the imported fire ant. Because of its efficacy of use in a bait formulation particularly attractive to fire ants, extremely small rates of application are required. As little as 4.2 g/ha (1.7 g/ac)* provides effective kill in an infested area. However, permanent eradication has not been possible with the control approaches used; the mobility and range of the winged fire ant queens result in reinfestation from neighboring areas.

Total mirex application through 1975 for imported fire ant control in the U.S. is estimated to have been no more than about 250,000 kg (550,000 lb). Although mirex has also been used on a smaller scale for control of the western harvester ant, the Texas leaf-cutting ant and the Hawaiian pineapple mealybug, the quantities have been small relative to those used for fire ants. Total production in the U.S. of mirex through 1975 was approximately 400,000 kg (800,000 lb), but it is estimated that approximately 150,000 kg (330,000 lb) of this was exported for pest control in other countries.

Kepone's main use has been as a pesticide for the banana root borer, outside of the United States. There are no large-scale agricultural uses in the U.S. Its principal use was around homes and buildings as an ant and roach poison in the form of baits or traps containing only 0.125 percent active ingredient. Total production of Kepone, from its initial manufacture in 1951 through its suspension in August, 1975, was about 1,600,000 kg (3,500,000 lb) of which from 90 to 99 percent appear to have been exported. (Much of this exported Kepone was apparently not used directly as a pesticide but was converted to other pesticidal compounds, e,g., Kelevan).

2.2 STRUCTURE AND PREPARATION

Mirex and Kepone are two representatives of the large family of cyclodiene insecticides synthesized from hexachlorocyclopentadiene. Mirex is a white, odorless crystalline compound composed entirely of carbon and chlorine atoms, with the empirical formula $C_{10}Cl_{12}$. In the United States, the common chemical name is dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta (cd) pentalene; the systematic name is dodecachloropentacyclo $5.3.0.0^{2,6}.0^{3,9}.0^{4,8}$ decane. The Allied Chemical Corporation used the name Dechlorane for the same compound utilized as a flame retardant in polymeric materials (Alley, 1973). Chlordecone, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta (cd) pentalene-2-one (trade names GC-1189 or Kepone) is the ketone analog to mirex, with the empirical formula $C_{10}Cl_{10}O$. Commercial Kepone is a white to tan solid which sublimes, with some decomposition, at about 300 C.

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*This small quantity has been further reduced by recent improvements in bait formulations. The 1977 program used a 0.1 percent bait at a rate of 2.5 kg/ha (1 lb/ac), equivalent to 0.45/g/ac of active ingredient. Experimental degradable baits containing even smaller concentrations (0.05 and 0.025 percent mirex) have been developed and are being tested.

An excellent discussion of the structures and preparative procedures for the insecticides of the diene-organochlorine group, including mirex and Kepone, is found in "Chlorinated Insecticides, Volume I", by Brooks (1974). Brooks notes that mirex can be prepared directly by the reaction of hexachlorocyclopentadiene ("hex") with itself in the presence of aluminum chloride, or by the reaction of Kepone with phosphorus pentachloride. The preparation and the cage structures of mirex and Kepone are shown graphically in Figure 2.1, from Brooks.

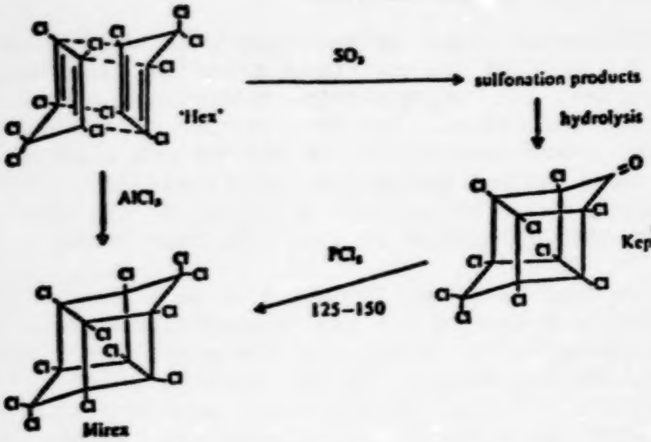


Figure 2.1 Synthesis of Mirex and Kepone

Source: Reprinted, with permission, from Chlorinated Insecticides, Vol. I. (c) The Chemical Rubber Co., CRC Press, Inc. (1974).

As described by Brooks (1974).

....."When hex is treated with sulfur trioxide, fuming sulfuric acid, chlorosulfonic acid or sulfuryl chloride at 35 to 80 C, it reacts with itself by addition to give a cage structure in which the allylic chlorine atoms of one molecule are substituted by hydrolyzable sulfur containing groups. The intermediate (or intermediates) loses sulfur on hydrolysis to give the corresponding cage structure (70 percent yield) containing a carbonyl bridge, which has been marketed as an insecticide under the trademark Kepone, also known as chlordecone (Figure 2.1). The technical product is more than 90 percent pure, and sublimes, with some decomposition, near 350 C."

"When heated with phosphorus pentachloride at 125 to 150 C, the keto group is replaced by two chlorine atoms to give mirex, a $C_{10}Cl_{12}$ chlorinated hydrocarbon (Figure 2.1), identical with the product formed directly from hex by heating it with aluminum chloride either without solvent or in methylene chloride, carbon tetrachloride, tetrachlorethylene, or hexachlorobutadiene".

2.2.1 Hexachlorocyclopentadiene

Hexachlorocyclopentadiene ("hex") is the precursor of both mirex and Kepone. It is also the starting material for numerous organochlorine insecticides, including aldrin, dieldrin, heptachlor, endrin, chlordane, endosulfan, and Pentac^{R}, and for a number of chlorinated compounds used to impart flame resistance to resins and plastics. Thus, production of hex has been large. Whetstone (1964) estimated 1962 production as at least 22.5 million kg (50 million lb) in 1962; Lu, et al., (1975) estimated 1975 production at about the same level.

Since hex is primarily utilized as a chemical intermediate, with no significant end uses of its own, essentially none of this toxic substance is intentionally dispersed into the environment. However, in view of the large production quantities the possibilities of loss of hex and its byproducts during manufacture and use cannot be disregarded. Additionally, unreacted hex is a potential contaminant in a number of the products made from hex.

During the course of the current study it became apparent that the environmental impacts of hexachlorocyclopentadiene and related compounds, heretofore seriously neglected, warranted further investigation. An investigation of hex is currently in progress which will be the subject of a separate report, to be issued soon. The present report considers only mirex and Kepone.

2.3 CHARACTERIZATION OF MIREX AND KEPONE

2.3.1 Physical and Chemical Properties

2.3.1.1 Mirex—

Mirex ($C_{10}Cl_{12}$) is a white, crystalline, free-flowing solid of molecular weight 545 whose preparation was first described by McBee, et al., (1956). Reported melting point was 485 C. According to Brooks (1974) mirex has a vapor pressure of 6×10^{-6} mm Hg at 50 C.

Mirex is fairly soluble in organic solvents. Brooks reported the following solubilities:

Solvent	Percent
Methyl ethyl ketone	5.6
Carbon tetrachloride	7.2
Benzene	12.2
Xylene	14.2
Dioxane	15.2

Alley (1973) summarized the solubilities of mirex as follows:

Solvent	g/100 ml	Solvent	g/100 ml
Ethyl alcohol	0.7	Cyclohexane	3.4
Acetonitrile	0.4	Isooctane	2.1
2-Butanone (MEK)	4.2	n-Hexane	2.8
Piperidine	11.3	Toluene	14.3
Morpholine	14.5	Carbon disulfide	18.1
Pyridine	10.1	Methylene	10.2
Diethyl ether	9.1	Chloroform	17.2
Tetrahydrofuran	30.3	Carbon tetrachloride	5.9

Mirex has a very low solubility in water, not exceeding 1 ppb (de La Cruz and Naqvi, 1973). Hollister, et al., (1975) reported evidence that 0.2 ppb mirex was the maximum solubility possible in seawater. Alley (1973) indicated even lower solubilities:

	Solubility, ppb
Water	1
Water, 0.5% salinity	0.25
Water, 2.5% salinity	0.11
Water, 4.0% salinity	0.04

Technical mirex has a high purity in comparison to a number of other chlorocarbon insecticides. Analysis of technical mirex for Kepone content by the Chemical and Biological Investigations Branch of EPA, Beltsville, Maryland, showed mirex content to exceed 95 percent, with only traces of Kepone present (U.S. Environmental Protection Agency, 1976).

Kepone Content of Technical Mirex

Matrix	Mirex Content, Percent	Kepone Content, ppmw
Technical grade mirex	95.19	2.58
Technical grade mirex in soybean oil	1.71	2.38
Technical grade mirex in soybean oil on corncob grits	0.26	0.25

2.3.1.2 Kepone—

As described in a Development Information Bulletin (Allied Chemical Corporation, 1958a), Kepone is a stable, white to tan solid which sublimes, with some decomposition, at about 300 C. It is readily soluble in acetone, lower aliphatic alcohols, and somewhat soluble in benzene, toluene, and hexane. It is nearly completely soluble in aqueous sodium hydroxide. Subsequent Allied Chemical Corporation data, reported by Smith (1976), indicated that the aqueous solubility is 1.5 to 2 ppm over the pH range of 4 to 6. In the 9 to 10 pH range, solubility increases to 5 to 70 ppm. In 0.001 M NaOH (pH 10.9) solubility is 176 ppm.

Upon exposure to ordinary temperatures and humidities, the anhydrous compound readily takes up water, up to three molecules of water. (Allied Chemical Corporation, 1958a). The trihydrate, $C_{10}Cl_{10}O \cdot 3H_2O$ (9.92 percent water) appears to be the stable form. Because the anhydrous form takes up moisture rapidly during mixing and milling steps, it was not considered satisfactory for dust bases and water-dispersible powders. On the other hand, the trihydrate was not considered to be entirely satisfactory for producing emulsifiable concentrates. However, compositions between the monohydrate (3.54 percent water) and the dihydrate (6.84 percent water) were regarded as satisfactory for both uses, so that total water specifications for technical Kepone were established at 3.5 - 6.0 percent (Allied Chemical Corporation, 1958b).

The average analysis of Kepone produced during the 1966 Allied Chemical Corporation semiworks production campaign was as follows (Smith, 1976):

	Percent by Weight
Water	5.2
Methanol insolubles	0.3
Hexachlorocyclopentadiene	0.1
Sulfate	0.03
Kepone	94.4

2.3.2 Chemical and Thermal Stability

Both mirex and Kepone possess a high degree of chemical and thermal stability; Kepone is somewhat less stable than mirex, presumably as a result of the replacement of two chlorine atoms with a carbonyl bond.

2.3.2.1 Mirex—

Mirex is notably stable. McBee, et al., (1956) reported it unaffected by the following: zinc dust and hydrochloric acid, acetic acid, or methanol; silver nitrate and ethanol for long periods; alkaline reagents such as potassium hydroxide in methanol or lithium hydride in ether; and oxidizing agents such as ozone, potassium permanganate, chromic acid, sulfur trioxide, sulfuric acid, or nitric acid. However, Dilling, et al., (1967) found that mirex reacted with lithium and tert-butyl alcohol to give a saturated hydrocarbon and endo-dicyclopentadiene.

There is general agreement that mirex is thermally very stable. In the pioneering work of McBee, et al., (1956) it was found that mirex underwent pyrolysis only at very high temperature (500 C or above), giving largely carbonaceous material and chlorine, with only a small amount of hexachlorocyclopentadiene. Eaton, et al., (1960) performed pyrolysis tests at 500 C in flowing nitrogen; the condensate was found to be unchanged starting material contaminated with small amounts of hexachlorocyclopentadiene. On the basis of more recent work, it is considered possible that vaporization was the predominant reaction occurring under these experimental conditions.

Holloman, et al., (1975) investigated the major thermal degradation products of mirex. These pyrolysis experiments were conducted by sealing 50 to 100 mg of analytical standard grade mirex in 10-ml Neutraglas ampules and heating them in a muffle furnace for 30 minutes at the selected temperature. After cooling the ampules were broken and the contents dissolved for analysis in pesticide grade hexane. No degradation was detected below 525 C, in agreement with McBee, et al., (1956) and Eaton, et al., (1960). At 525 C, the principal product was hexachlorobenzene, with traces of hexachlorocyclopentadiene. A sample heated to 550 C in an ampule lost on the average, 15 percent of its original weight. The residue remaining was found to be 72 percent hexachlorobenzene, verified by mass spectrometric, infra red (IR), ultraviolet (UV), and gas-liquid chromatographic (GC) analyses. Other tests indicated that hexachlorocyclopentadiene, postulated as the most probable mode of fragmentation, exists only a short time at pyrolysis temperatures, and is rapidly converted to hexachlorobenzene.

In recent research on suitable methods for the disposition of unwanted stores of Kepone and Kepone-contaminated materials, high-temperature controlled incineration has been investigated by Duvall and Rubey (1976), under the sponsorship of the EPA. Both mirex and Kepone were included in the study, and new insight on their thermal stabilities has resulted from this investigation.

The objective of the study was to obtain laboratory test data which could be applied to large-scale thermal destruction of Kepone and similar pesticides, using oxidative rather than pyrolytic conditions. Tests were conducted at a series of temperatures ranging from 250 to 900 C. Pure pesticide was vaporized at a low temperature, 200 to 300 C, and the vapor was passed through a high-temperature quartz tube in a furnace at constant temperature; residence time (1 second) was controlled by the rate of air flow. The effluent from each test was trapped and subsequently analyzed by gas chromatography. Results of the mirex tests are shown in Figure 2.2.

In agreement with the findings of previous investigators, mirex decomposition began to occur in the 550 to 600 C range, and was rapid at 700 C. In companion thermogravimetric analyses, vaporization commenced at about 140 C and was complete by 230 C. Two principal degradation products were observed. One, whose concentration peaked at about 700 C, was determined by GC to be hexachlorobenzene (peak Mc). The second, observed only in the test at 600 C, was hexachlorocyclopentadiene. On the basis of the analogous Kepone experiments, it appears that the decomposition temperature for mirex probably overlaps that for hex to the extent that the hex peak essentially disappears.

Mirex is also notably resistant to photolytic decomposition in the environment. Gibson, et al., (1972) have conducted both field and laboratory experiments. Radioactively-labelled ^{14}C-mirex deposited on thin layers of silica gel (Eastman Chromagram Sheet No. 6016) was exposed to sunlight in the environment. After three months of exposure, the gel was extracted with methanol and the extracts were analyzed by GC and TLC (thin-layer chromatography). The principal photoproduct comprised only 5 percent of the total persisting residue. The major mirex photoproduct was also prepared in the laboratory by irradiating silica gel deposits with artificial light (fluorescent sunlamps) at a lamp to subject distance of 7 cm for 48 hrs. The compound prepared by artificial light exhibited identical behavior on TLC and GC with the product from actual environmental exposure. Although both procedures indicated one or more minor photoproducts, the remaining residue consisted almost exclusively of unchanged mirex.

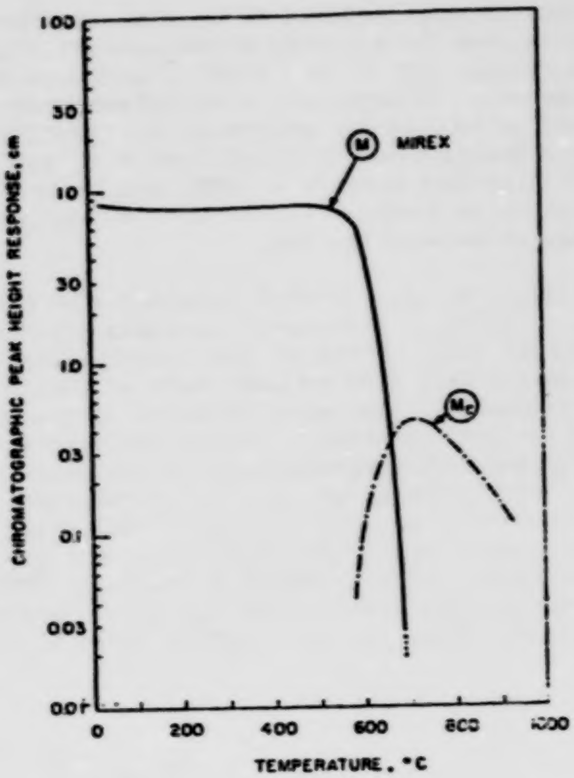
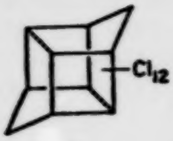
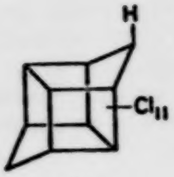


Figure 2.2 Thermal destruction plot for mirex. Adapted from Duvall and Rubey (1976).

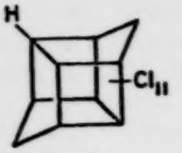
Evidence suggested that the photoproduct was a monohydro derivative of mirex. The symmetry of the carbon nucleus of mirex (Structure I) permits only three isomeric monohydro derivatives:


Cl_{12}

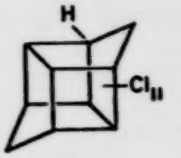
I


H
Cl_{11}

II


H
Cl_{11}

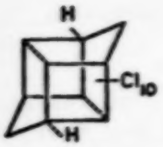
III


H
Cl_{11}

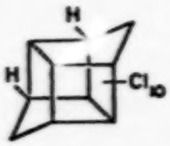
IV

Compound II was prepared by lithium-aluminum hydride reduction of Kepone and subsequent chlorination of the alcohol with phosphorus pentachloride by Dilling, et al., (1967), and its structure was determined unambiguously. In addition, a second monohydro isomer was produced but it was not possible to determine whether its structure was III or IV. Gibson's photoproduct differed from II on the basis of TLC, GC, IR, and proton magnetic resonance (PMR) analysis. Thus, the photoproduct was deduced to be either III or IV, although it was not possible to distinguish between the two.

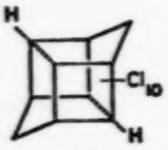
Alley, et al., (1973) obtained similar results upon irradiating mirex, (0.04 M) in hydrocarbon solvents (cyclohexane or isooctane) with a mercury UV lamp. After 48 hrs irradiation 95 percent of the mirex had disappeared, with a 40 percent yield of photoproducts. Of the two major photoproducts, the mass spectra indicated one to be a monohydro derivative and the other a dihydro derivative. Structure II was ruled out for the monohydro photoproduct on the basis of GC and IR analyses. Alley et al., (1973) and Alley, et al., (1974a) have assigned Structure III to the monohydro photoproduct. Photolysis of the purified monohydro photoproduct revealed that it was a precursor of the dihydro photoproduct. Nuclear magnetic resonance (NMR) and IR analyses suggested that the dihydro derivative has more symmetry than the monohydro derivative. Alley, et al., (1974a) assigned structure VII for the dihydro compound.



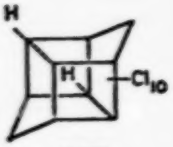
V



VI



VII



VIII

Alley, et al., in a later paper (1974b) cited research by Zijp and Gerding (1958) which showed that mirex has virtually no absorption at wavelengths greater than 250 nm, and thus concluded that very little photochemical degradation by sunlight would be expected. This is consistent with the results of Gibson, et al., (1972) and of Carlson, et al., (1976). However, work of Alley, et al., showed that the degradation of mirex by sunlight was significantly enhanced by its interaction with aliphatic amines, e.g., triethylamine.

The photodecomposition of mirex in aliphatic amines proved to be different from that observed in hydrocarbon solvents in two important aspects, the product obtained and the wavelength of light absorbed. The major photoproduct (verified by its IR spectrum) was the monohydro derivative, II. A second monohydro photoproduct, III, was formed in smaller quantities. In addition, a mixture of dihydro products was also formed.

The wavelength of light required for the reaction was different in amines from that in hydrocarbons. Sunlight or sunlamp caused rapid decomppsition of mirex in amines, but no reaction could be detected under similar conditions when the solvent was cyclohexane. The enhancement of decomposition is attributed to a charge transfer complex between the amine and mirex. Similar observations of photodecomposition of pesticides promoted by aromatic amines have been reported by others.

It was suggested by Alley, et al., that it might be possible to devise photodegradable formulations of mirex by the incorporation of amines into the bait used for control of the imported fire ant. Holden (1976) indicated that this approach was being followed up. More recent information (Alley, 1977) disclosed that a considerable degree of success has been achieved. Baits which are still attractive to fire ants but which decompose in the environment in 4 to 6 weeks have been developed and field tested. Efficiency of the new type of bait is good, even at low application rates. Decomposition proceeds both photolytically and thermally; at 30 C photolytic decomposition by sunlight is 3 to 4 times faster than thermal decomposition. Thermal decomposition is enhanced by the addition of ferrous chloride. Indications are that the toxicity of the successive degradation products decreases from monohydro to dihydro to trihydro. Toxicity studies will be necessary to determine the overall safety of this new type of bait , but it appears to possess considerable advantage over the original mirex bait.

Some of the most definitive evidence of the exceptional stability of mirex in the environment was recently reported by Carlson, et al., (1976), who investigated two field locations where unusually large amounts of mirex were deposited and left for 12 to 5 years. The first location was near Gulfport, Mississippi, where mirex (on attapuulgite clay) was applied in 1962 to experimental plots in a fire ant control test at the rate of 1.12 kg mirex/ha (1 lb/ac). Present dosage is at a recommended rate of 1.7 or 3.4 g mirex/acre (4.2 to 8.4 g/ha). Soil cores and samples of the loose, sandy soil were gathered from three plots in 1974 and held in sealed metal cans until extraction.

The second location was near Sebring, Florida, where an airplane carrying granulated 4X type mirex bait crashed in 1969 at the edge of a small shallow pond, dumping its entire load of bait into the pond. The resulting layer of bait which contained 0.3 percent of mirex (3000 ppm) covered an area of approximately 3 m in diameter and about 0.25 meter deep, in a location usually under water about 0.2 to 0.5 m. The water has been observed to recede in the spring, exposing the bait to sunlight. Samples of bait and the underlying muck were collected in March, 1974 and analyzed by GC, TLC, and gas chromatography-mass spectrometry (GC-MS).

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*Designated as Ferriamicide

Analysis by GC of crude extracts showed detectable quantities of two monohydro products, two dihydro products, and Kepone. In the Gulfport samples the concentration of mirex plus degradation products represented about 50 percent of the mirex applied 12 years before. Kepone represented 3 to 6 percent of the total residues; unchanged mirex ranged from 65 to 73 percent. Similarly, in the Sebring samples, mirex ranged from 76 to 81 percent and Kepone comprised 1 to 6 percent (Table 2.1). Identity of the decomposition products was verified by GC, by computer-reconstructed gas chromatograms (RGC), and by limited mass chromatograms (LMS) from stored chemical ionization spectra.

The authors note that this decomposition sequence is likely to occur at any location where mirex bait is deposited on the soil, and that although Kepone is the second most abundant product observed (up to 10 percent as much as the recovered mirex) it is just one of a succession of products formed by exposure of mirex to light. Thus, this demonstrates a pathway by which mirex can disappear from the environment.

Lane, et al., (1976) presented the results of a study designed to assess the possible alteration of mirex during food processing via UV or gamma radiation. When eggs from mallard ducks, *Anas platyrhychos*, which had been fed diets containing mirex at levels of 1 and 100 ppm for 25 weeks were subjected to UV and gamma radiation, a number of degradation products were observed. Seven derivatives were obtained from photolysis, and eight from gamma irradiation; results of combined gas chromatography--mass spectrometry are summarized in Table 2.2. Mirex residues after UV radiation (1 ppm mirex diet) were:

Control, ppm	2.48±0.05
24 hours, ppm	2.04±0.04
48 hours, ppm	1.60±0.07

Mirex residues after various dosages of gamma radiation (100 ppm mirex diet) were:

Control, ppm	232.00±12.84
1000 rad, ppm	206.99± 7.63
2000 rad, ppm	163.52± 4.80
3000 rad, ppm	130.96± 3.14
4000 rad, ppm	85.45± 1.41

Photolysis caused a 36 percent decrease in mirex at 48 hours whereas gamma irradiation at 4.5 M rad caused a 64 percent decrease. A linear relationship existed between loss of mirex and time for both UV and gamma irradiation. Peak 7, the major photolytic and gamma irradiation product of mirex, corresponds to the major monohydro photolysis product obtained by Alley, et al., (1974a), and Peak 8 corresponds to the compound obtained more recently by these same investigators by photoreduction of mirex in triethylamine (Alley, et al., 1974b). The compounds of peaks 5 and 6 were found to be isomeric and unsymmetrical dihydro derivatives of mirex, with Peak 7 being the precursor compound. Peak 4 proved to be a

TABLE 2.1. COMPOUNDS AND CONCENTRATIONS FOUND IN RECOVERED SOIL AND BAIT SAMPLES AFTER 5 AND 12 YEARS[a]

		Gulfport Samples, ppm[c]			Sebring Samples, ppm[d]	
Compounds	R_t[b]	Plot 27	Plot 42	Plot 70	Surface Bait[e]	Muck
Mirex	1.00	0.497	0.310	0.192	633	0.206
Monohydro	0.76	0.014	0.015	0.010	39	0.013
Monohydro	0.67	0.110	0.092	0.054	65	0.020
Dihydro	0.52	0.019	0.010	0.012	14	0.012
Kepone	0.48	0.021	0.026	0.019	10	0.015
Dihydro	0.42	0.021	0.018	0.008	17	0.005
Total		0.682	0.471	0.295		0.271

[a] Source: Carlson, et al, (1976), reprinted with permission from Science (C) Amer. Assoc. for the Advancement of Science (1976).

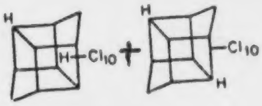
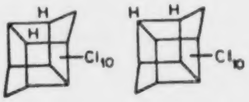
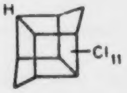
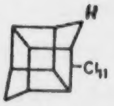
[b] Retention time relative to mirex (1.00) on a DC-200 column.

[c] Taken 12 years after application.

[d] Taken 5 years after initial dispersion.

[e] Bait mixed with soil in unknown proportion.

TABLE 2.2 UV AND γ IRRADIATION PRODUCTS OF MIREX[a]

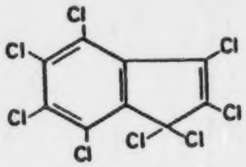
Peak	Retention time relative to Mirex, 10% DC 200 at 195 C	Products
1	0.30	Not identified
2	0.32	Not identified
3	0.37	Not identified
4	0.42	
5	0.48	
6	0.51	(Peaks 5 and 6)
7	0.65	
8	0.75	 (obtained in γ radiation only)
9	1.00	Mirex (starting material)

[a] Source: Lane, et al (1976). Reprinted, with permission from J. Agricultural and Food Chemistry. (c) American Chemical Society (1976).

mixture of symmetrical dihydro compounds similar to those otained by Alley, et al., from photolysis of mirex in cyclohexane. No mass spectra were obtained on Peaks 1, 2, or 3 because of insufficient samples.

2.3.2.2 Kepone—

The thermal and chemical stability of Kepone has been much less thoroughly studied. Eaton, et al., (1960) performed pyrolysis tests on Kepone similar to those on mirex (Section 2.3.2.1). As a result of the lower decomposition temperature of Kepone, quantities of decomposition products were observed after exposure to 500 C. The principal pyrolysis product (80 percent yield) was octachloroindene, C_9Cl_8:



Small quantities of hexachlorobenzene and carbon tetrachloride were also detected along with trace amounts of hexachlorocyclopentadiene. Kecher, et al., (1969) confirmed these findings, reporting that when Kepone was held in a sealed ampule at 250 to 300 C for 1 hr, hexachlorocyclopentadiene and a degradation product with the nominal composition of C_9Cl_8 were detected in the pyrolysis products.

Duvall and Rubey (1976) have extensively examined the high-temperature destruction of Kepone and related pesticides; their findings on mirex are discussed in Section 2.3.2.1. Kepone vaporizes and decomposes at lower temperatures than mirex. Thermogravimetric analysis in a flowing air stream showed vaporization commencing at about 95 C and being essentially complete at 195 C. An early weight loss was observed between 30 and 50 C, presumably due to the loss of water of hydration. Under the standard test conditions (~1 second residence time), Kepone decomposition began to occur at temperatures above about 350 C, and was rapid at 450 to 500 C (Figure 2.3). Hexachlorobenzene (peak K_c) was prominent at temperatures below 600 C, hexachlorocyclopentadiene (peak K_a) was formed at temperatures below 500 C, and was not found at temperatures above 600 C. Carnes (1977a) states that the intermediate peak (K_b) was unidentified. The planned addition of GC-MS to the instrumentation is expected to provide identification of this degradation product in subsequent studies (Carnes, 1977b). The significant yield of octachloroindene reported by Eaton, et al., (1960) and confirmed by Kecher, et al., (1969) may explain the unknown peak.

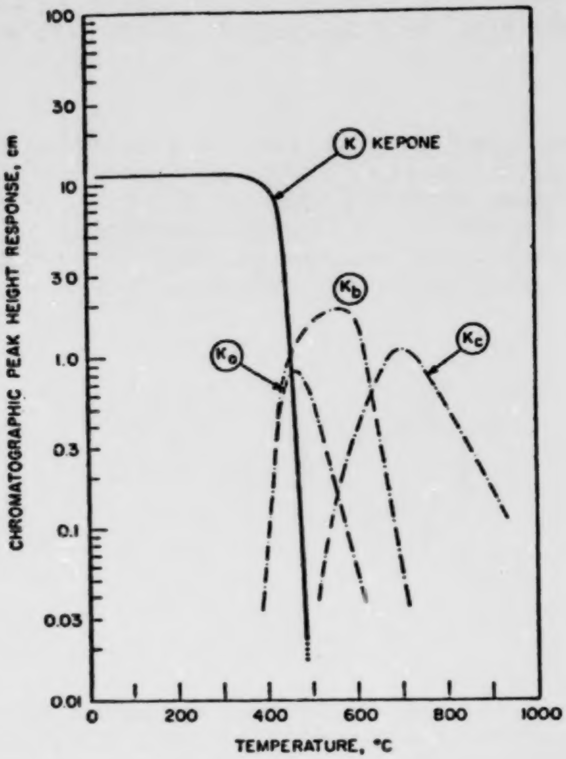


Figure 2.3 Thermal destruction plot for Kepone
Source: Duvall and Rubey (1976)

When 40 micrograms of Kepone vapor, in an excess of flowing air, was subjected to a 900 C environment for approximately 1 second, essentially only hexachlorobenzene was found in the effluent, and only at nanogram levels. Residual Kepone trapped was of the order of 100 to 200 picograms (Duvall and Rubey, 1976). The effect of residence time on Kepone decomposition at a lower temperature was also investigated in a series of tests at 433 C. Destruction increased from less than 10 percent at 0.3 second to about 70 percent at 2 seconds (Figure 2.4).

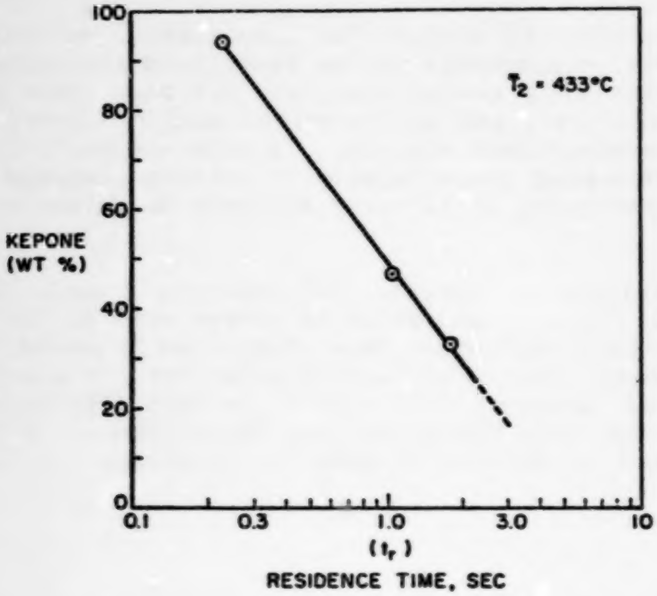


Figure 2.4. Effect of residence time on thermal destruction of Kepone

Source: Duvall and Rubey (1976).

2.4 ANALYSIS

2.4.1 Methods of Analysis

Analytical methods for mirex and Kepone are similar to those used for other chlorinated pesticides. These methods for mirex and Kepone can be grouped into macromethods suitable for analysis of large quantities of material, and micromethods suitable for analysis of residues in the environment at trace or near-trace concentrations. The classical macroanalytical techniques (e.g., gravimetric, polarographic, spectrophotometric analyses) useful for product quality control purposes, are not suitable for residue analysis, and are omitted from the following discussion. Only the microanalytical methods appropriate for assessing the environmental effects of these compounds and their degradation products are considered here.

2.4.1.1 Gas-Liquid Chromatography--

GC is the preferred method for quantitative determination of pesticides and related compounds in the Pesticides Analytical Manual, Vol. I. (Food and Drug Administration, U.S.Department of Health, Education, and Welfare), and is the method used for nearly all the analytical determinations contained in this document. It is often supplemented by thin-layer chromatography to confirm compound identity, or by mass spectrometry or infrared analysis to determine compound structure.

In gas-liquid chromatographic (GC) analysis a small quantity of sample is injected into a column which is packed with an inert carrier coated with a suitable stationary phase. When a gas is passed through the column the materials are partitioned between the gas phase and the stationary phase. Brooks (1974) points out that this separation is similar to fractional distillation and that the performance of the column in separations can be defined in terms of theoretical plate content (n) by the equation

$$n = 16(Rt/W)^2$$

where Rt = retention time (distance between solvent peak and sample peak, expressed as time).

W = sample peak base width, expressed as time.

The materials used to carry the stationary phases are by no means always inert and usually must be treated in various ways to remove reactive centers which may decompose the samples. Many methods have been reported for the preparation of column-packing materials, and a large number of stationary phases suitable for various situations in organochlorine pesticide analysis have been described in the pesticide literature. Retention time is the principal parameter used to discriminate between compounds, and tabulations have been compiled for various stationary phases, most commonly referenced to aldrin (R_t = 1.000). (Burke, 1966; Pesticide Analytical Manual, Vol. I). Both Kepone and mirex have long retention times relative to aldrin, approximately 2.7 for Kepone, and 5.0 for mirex; very few pesticides have longer retention times than mirex.

Since about 1960, with the development of the microcoulometric detector by Coulson and the electron-capture detector by Goodwin, GC has achieved both sensitivity and selectivity for organochlorine compounds (Brooks, 1974).

Accurate analyses with GC critically depend on careful techniques and rigid control of operating parameters. Sample solutions must be cleaned up (i.e., separated from coextractives) sufficiently to permit identification and measurement of residues and to prevent contamination or destruction of any part of the GC system. The column and/or the detector may be impaired by the injection of insufficiently cleaned-up samples, especially when the method and the gas chromatograph are being used repeatedly. (Pesticide Analytical Manual, Vol. I).

Cleanup for the AOAC (Association of Official Analytical Chemists) methods, detailed in the Pesticide Analytical Manual, is based, in general, upon extraction of pesticides from a sample of non-fatty substrates by acetonitrile or water-acetonitrile (preceded by a petroleum ether extraction for fatty or oily samples). The extracted sample is transferred to a dry hydrocarbon solvent e.g., petroleum ether, and is isolated from coextracted substances by chromatography on a Florisil column. The Florisil column is eluted with mixed petroleum and ethyl ethers or with a methylene chloride: hexane: acetonitrile system. These procedures are effective in removing normal contaminating substances from samples prior to GC analysis.

However, one serious problem with mirex determination by GC is the interferfence of Arochlor 1260^R*, which has a peak almost overlapping the mirex peak. Special sample cleanup procedures are required to eliminate this interference (Markin, et al., 1972). Arochlor 1254^R also interferes.

Polychlorinated biphenyls have been, up to now, very widely used chemicals. Their large-scale manufacture, about 275 million kg (600 million lb), in the U.S. between 1960 and 1970 has resulted in the inadvertent introduction of enormous amounts of PCB's into the environment (Su and Price, 1973). Reynolds (1969) noted that this was evidently first detected by Jensen in wildlife tissues in Sweden in 1966. Since then, PCB's have been found in wildlife and humans in most of the industrialized areas of the world (Su and Price, 1973), and the presence of PCB is a possibility in almost any environmental sample being analyzed for an organochlorine pesticide.

Since there are about 210 PCB isomers, with varying boiling points and GC retention times, they can interfere in the GC determination of virtually all organochlorine pesticides. This problem has given rise to much controversy in pesticide analysis, and may be the principal reason for the difficulty in recognizing PCB's as environmental contaminants (Su and Price, 1973).

The standard Florisil cleanup procedure does not effectively remove interfering PCB's and additional cleanup and separation procedures are required. Most depend on the use of a silicic acid column following the Florisil colummn (Armour and Burke, 1970: Biros, et al., 1971); Snyder and Reinert (1971) used a silica gel column directly on hexane extracts. Markin, et al., (1972) recommend a modification of Armour and Burke's

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*Arochlor is the tradename of a series of polychlorinated biphenyls formerly manufactured by the Monsanto Chemical Company. Their manufacture in the U.S. has recently be terminated.

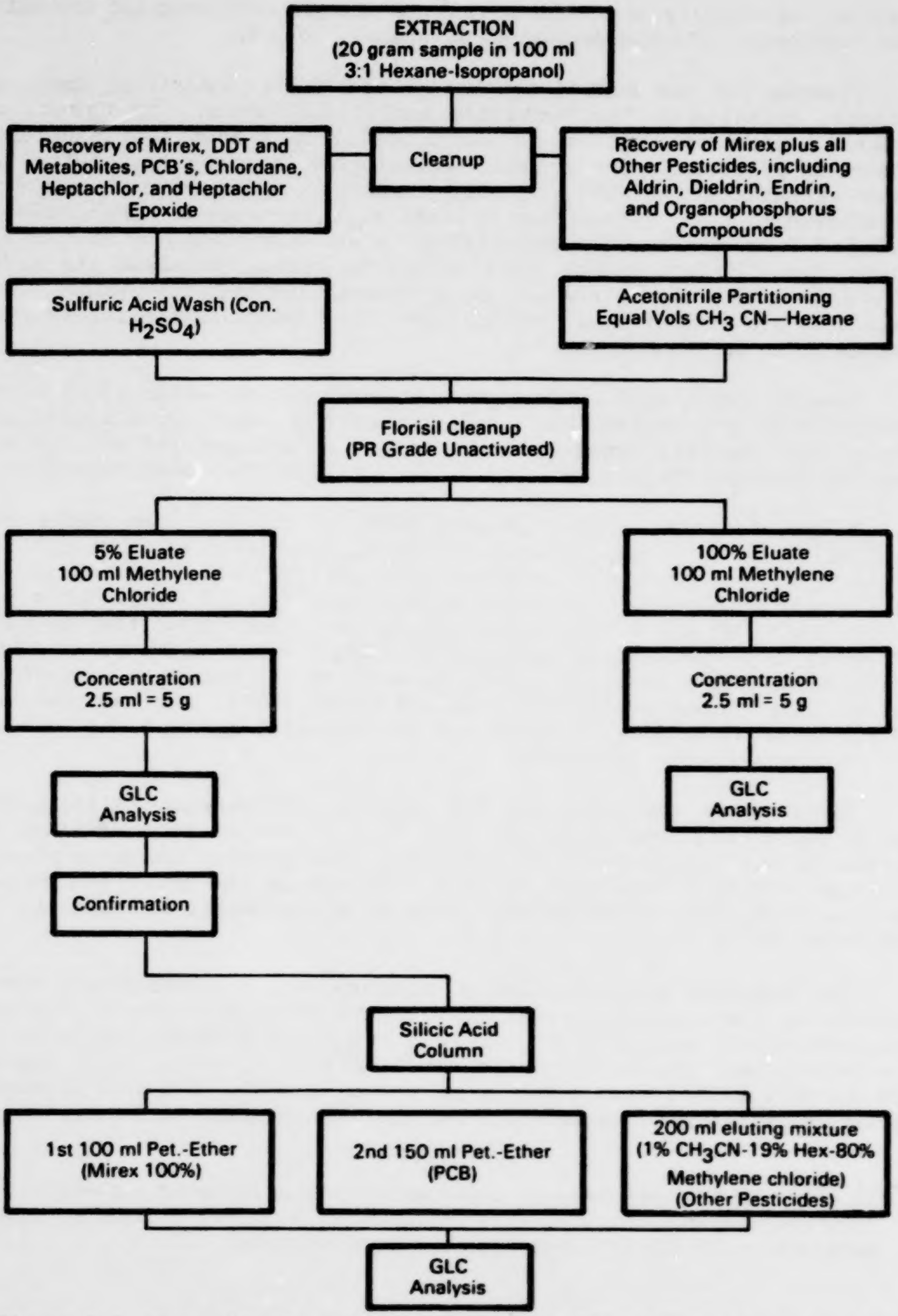
EXTRACTION (20 gram sample in 100 ml 3:1 Hexane-Isopropanol)
Cleanup
Recovery of Mirex, DDT and Metabolites, PCB's, Chlordane, Heptachlor, and Heptachlor Epoxide
Recovery of Mirex plus all Other Pesticides, including Aldrin, Dieldrin, Endrin, and Organophosphorus Compounds
Sulfuric Acid Wash (Con. H_2SO_4)
Acetonitrile Partitioning Equal Vols CH_3 CN—Hexane
Florisil Cleanup (PR Grade Unactivated)
5% Eluate 100 ml Methylene Chloride
100% Eluate 100 ml Methylene Chloride
Concentration 2.5 ml = 5 g
Concentration 2.5 ml = 5 g
GLC Analysis
GLC Analysis
Confirmation
Silicic Acid Column
1st 100 ml Pet.-Ether (Mirex 100%)
2nd 150 ml Pet.-Ether (PCB)
200 ml eluting mixture (1% CH_3CN-19% Hex-80% Methylene chloride) (Other Pesticides)
GLC Analysis

Figure 2.5 Generalized analytical procedure flow diagram for mirex in biological samples. Adapted from Markin, et al. (1972).

method developed by Gaul and Cruz-La Grange (1971), which elutes mirex in the first silicic acid column fraction, PCB's in the second, and the remaining chlorinated hydrocarbon pesticides in the last (acetonitrile:hexane:methylene chloride) eluate. The generalized scheme used by Markin, et al., (1972) for biological samples, including the silicic acid cleanup, is illustrated in Figure 2.5. Porter and Burke (1973) proposed the use of unactivated Florisil followed by chromatography on an activated Florisil column (methylene chloride:hexane eluant). Bong (1975) has suggested a similar method.

Since it is not always evident in the literature on the analysis of environmental samples for mirex whether or not the possibility of PCB interference was recognized and compensated for, it should be recognized that possibilities for error exist. Markin, et al., (1972) noted that two possible cases of mistaken identity have appeared in the literature, in which mirex reported in seafood could have been either PCB's or Dechlorane.

PCB's can be eliminated by additional sample cleanup with methods such as those described above, but since Dechlorane is chemically identical with mirex, no unequivocal differentiation between the two is possible. Presumptive differentiation can be made on the basis of other evidence and deductive reasoning. The reported detection of $C_{10}Cl_{12}$ in the Great Lakes and in Great Lakes biota (e.g., fish and herring gulls) almost certainly resulted from the manufacture of mirex or Dechlorane and not from the fire ant control program in the southeastern U.S. (see Section 2.5.1.2). Similarly, the reported detection of mirex in the marine environment on the Pacific Coast is suspect, since it had no important application in that area, and was not reported to have been used there at all prior to 1971. Markin, et al., (1972) suggest that PCB's or Dechlorane are the cause of these reported mirex findings.

Lewis, et al., (1976) have described an alternative method for the determination of mirex in the presence of PCB's. The procedure depends on diethylamine--or triethylamine-assisted photodegradation of interfering PCB's prior to measurement of the mirex by electron-capture gas chromatography. A 275-W sunlamp with spectral output greater than 280 nm may be used as the irradiation source. Reductive dechlorination of the PCB apparently results through primary photoexcitation of the biphenyl, followed by hydrogen abstraction from the alkyl and amino groups of the amine. The method has been successfully applied to human tissue extracts for the presence of Arochlor 1260 and other commonly occurring pesticides, including dieldrin, heptachlorepoxide and DDT.

The same general GC procedures applicable to mirex are also applicable to the determination of Kepone; no mention has been observed in the literature of specific PCB interferences with Kepone analysis.

2.4.1.2 Thin-Layer Chromatography--

TLC is the method of choice for qualitative confirmation of chlorinated pesticide assays by gas chromatography and may be used as a semi-quantitative method of analysis if GC is not available. The methods and techniques of TLC have been proven reliable, reproducible, and rapid, and give results which can be reported semi-quantitatively (Pesticide Analytical Manual, Vol. I).

TLC involves partitioning of substances on thin layers of silica gel or alumina on plates of metal or glass. Cleaned up sample extracts and reference organochlorine pesticide standards are spotted on coated plates and developed with appropriate organic solvents. They are then visualized by exposing silver nitrate-sprayed plates under UV light. The Pesticide Analytical Manual indicates that the use of silica gel has been discontinued for routine thin-layer chromatography of chlorinated pesticides, as aluminum oxide possesses superior sensitivity.

2.4.1.3 Mass Spectrometry--

In his thorough discussion of chlorinated insecticides Brooks (1974) noted that GC separation with mass spectrometric examination of the separated fractions is undoubtedly the most valuable combination currently available to the residue analyst or toxicologist attempting to identify minute traces of organochlorine or other pesticides. In some cases, the mass spectrometer is able to provide analytical data from sample peaks not many orders of magnitude greater than the limits of comfortable detection afforded by the most sensitive GC-electron capture combinations. Recent publications on organochlorine compounds, particularly environmental studies, usually contain data on mass spectral fragmentation patterns. The techniques have been especially valuable for identifying some of the molecular rearrangement products of cyclodiene molecules.

Mass spectrometry is appropriately used when other analytical methods of adequate sensitivity and specificity are not available. Mass spectrometry, particularly in combination with gas chromatography (GC-MS) and isotope-labelled internal standards, can form the basis of a definitive quantitative assay for validating other assays. However, the disadvantage of GC-MS is that sample throughput is relatively slow, and that the system is difficult to fully automate (Foltz, 1977).

Since the large amount of data generated in a typical GC-MS analysis are difficult to manipulate the present trend is to couple GC-MS with a computer. Computer-based GC-MS analyses can be grouped into two categories: repetitive scanning or selected ion monitoring. In the former, the mass analyzer repetitively scans over the mass range of interest. Upon completion of the run, the computer reconstructs a total ionization chromatogram (TIC) by plotting the summation of ion intensities for each scan number. The plot is similar in appearance to a normal gas chromatogram and is designated a computer-reconstructed gas chromatogram (RGC). Its primary use is to indicate which scans contain mass spectral data corresponding to each component of interest. Selected ion monitoring, plotting the ion current at selected masses versus time or spectrum number, enables the mass spectrometer to be used as an extremely selective and sensitive detector (Foltz, 1977).

Both of these techniques are quite useful in confirming the presence of mirex and Kepone determined by gas chromatography. Perhaps even more valuable is their capability for determining molecular structures of the complex degradation products resulting from environmental exposure.

2.4.2 Analytical Considerations

The importance of removing PCB's before GC analysis for mirex has already been discussed. Inadvertent sample contamination with chlorine-containing compounds must be avoided, thus, metal cans rather than plastic bags are preferred for storage of soil samples. Samples not to be analyzed immediately, especially biota samples, are generally kept frozen until analysis. Reagents and solvents used in GC analysis must also be free of chlorine contaminants; and distilled-in-glass solvents are generally recommended.

Residues of mirex detected in the environment may be as low as 1 ppt in water, 1 ppb in soil, and 0.01 to 0.1 ppm in most animals. Thus, exceeding care must be taken throughout the handling and analysis of such samples to avoid biasing the results from artifacts.

No mention in the literature on PCB interference with Kepone analysis has been noted; when electron capture GC analysis is being employed, chlorine contamination of the sample must be avoided.

2.5 MANUFACTURE AND USE

2.5.1 Mirex

2.5.1.1 Manufacture--

Official statistics on the annual production of mirex are not available. All of the mirex produced in the U.S. is believed to be manufactured or distributed by the Hooker Chemicals and Plastics Corporation, Niagara Falls, New York (Markin, et al., 1972; Shapley, 1971). For a number of years, the same compound was sold by Hooker for nonagricultural uses under the name Dechlorane. The principal use was as a flame retardant for polymeric materials. Hooker still manufactures a line of products called "Dechloranes", but these are not $C_{10}Cl_{12}$ (Chambers, 1976). However, many of these current Dechlorane products also appear to be highly chlorinated diene-type compounds possessing many similarities to $C_{10}Cl_{12}$.

From 1959 to June, 1967, Hooker produced the chemical $C_{10}Cl_{12}$, and ground, packaged, and sold the finished product. The material was made and handled exclusively at the Hooker plant at Niagara Falls, New York. Beginning in June, 1967, Hooker purchased the material from other producers, but continued to grind, repackage, and sell the material. Sources of purchased material were Hexagon Laboratories, New York, New York (22,680 kg in 1966), and the Nease Chemical Company, State College, Pennsylvania, and Salem, Ohio (113,400 kg in 1966; 324,430 kg in 1968-69; and 250,000 kg in 1973-74). (Chambers, 1976).

Total production data are not available. Sales data for 1959-1975, separated into mirex and $C_{10}Cl_{12}$ sales, have been reported by Hooker to EPA (Table 2.3). As shown by this tabulation, nearly three-quarters of the $C_{10}Cl_{12}$ sold was used for nonagricultural purposes during this 17-year period; mirex represented only about one-quarter of the sales. The principal nonagricultural use of $C_{10}Cl_{12}$ was to impart flame resistance to polymeric materials. As noted by Hutzinger, et al., (1976) most polymers, with the exception of those containing high percentages of chlorine or fluorine, are relatively easily flammable. With increasingly strict regulations on the flammability of clothing and home furnishings, annual consumption of flame retardants can be expected to increase. Hutzinger, et al., (1973) quote one estimate of 20,000 to 40,000 tons by 1980.

Some of the reactive type flame retardants can be incorporated into the polymer, either by copolymerization or by chemical reaction with a reactive group of the polymer, e.g., chlorendic anhydride, and 2,5-dibromoterephthalic acid (Hutzinger, et al., 1973). Dechlorane, however, falls into the additive type, incorporated by physical blending with the polymer. The halogenated fire-retardant additives such as Dechlorane have some advantages over the chlorinated paraffins and halogenated polyphenyls because they do not cause reductions in thermal properties; in fact, the result is often an increase in the heat resistance of the flame-retardant composition. Dechlorane, with a chlorine content of 78 percent, and a melting point of 485 C, was one of the first of these additives.

Such physically blended compounds would be expected to be more prone to leaching or escape from the finished polymer product. Even chemically-combined flame retardants would be susceptible to release upon incineration, a common ultimate fate for many organic polymers. Thus, additional investigation into the environmental fate of Dechlorane-treated polymers appears to be needed, in view of its much greater use than that of mirex, and especially because of the reports of mirex in the environment in sections of the U.S. where mirex itself has been employed only at a very minor level or not at all.

Data are unavailable on the total use of mirex as a pesticide in the United States. However, compared with other chlorinated pesticides, mirex was a small-volume pesticide. In 1972, a year for which estimates are available for consumption of several other chlorinated pesticides (U.S. Environmental Protection Agency, 1975), mirex sales compared as follows:

TABLE 2.3 SALES OF $C_{10}Cl_{12}$, 1959 - 1975[a]

	Sales of $C_{10}Cl_{12}$, kg		
Year	As Mirex	For Nonagricultural Use	Total
1959	--	50	50
1960	--	800	800
1961	90	4000	4.090
1962	5400	35,900	41,300
1963	11,600	129,200	140,800
1964	12,700	210,000	222,700
1965	24,500	245,700	270,200
1966	31,300	150,200	181,500
1967	32,900	72,300	105,200
1968	28,100	126,300	154,400
1969	46,300	32,800	79,100
1970	26,300	20,700	47,000
1971	13,600	37,200	50,800
1972	61,500	54,400	115,900
1973	51,300	--	51,300
1974	36,300	1,300	37,600
1975	18,200	4,600	22,800
Total	400,090	1,125,450	1,525,540
Percent	26.2	73.8	100.0

[a] Source: Chambers (1976).

Pesticide	Metric Tons
Toxaphene	31,000 - 43,000
DDT	20,400
Aldrin	5,400
Mirex	61.5

2.5.1.2 Use--

The imported fire ant is generally believed to have first been inadvertently introduced into the U.S. at the port of Mobile, Alabama, in 1918. It did not spread rapidly at first, but spread widely throughout the southestern United States in the 1950's. The present distribution of this pest is shown in Figure 2.6. The boundary of -6 to -12 C (10 to 20 F) minimum annual temperatures, which appears to prevent further northward spread, is also shown. Although the West Coast of the U.S. is presently free of the imported fire ant, annual temperatues there would be conducive to its spread to that area.

In areas where the fire ant is established, a concentration of about 75 to 100 mounds per hectare (30 to 40 per acre) is usual. The mounds range in size from those that are barely visible to mounds that might be 1 m in diameter and almost 1 m tall. When the mound of an established colony is disturbed, thousands of ants pour out, seeking their attacker. (Alley, 1973).

The Federal government and the states began their effort to combat the fire ant in 1957, initially by spraying dieldrin at a rate of 2.24 kg/ha (2 lb/ac). Heptachlor at a rate of 1.12 to 1.25 kg/ha (1.00 to 1.25 lb/ac) was substituted in 1958; this dosage was later reduced to two applications at a rate of 0.28 kg/ha (0.25 lb/ac), spaced 3 or 4 months apart (Coon and Fleet, 1970).

In 1961, mirex was developed by the Allied Chemicals and Plastics Corporation for control of several ant species. Following the development of an effective bait, the first field test was made near Gulfport, Mississippi, in the fall of 1961, (Markin, et al., 1972). Since that date, mirex bait has totally supplanted the earlier insecticides for fire ant control by area application because of its superior effectiveness and its drastically lower rates of application to the environment.

The mirex bait used for fire ant control consists of ground corn cob grit (85 percent), once-refined soybean oil (approximately 15 percent), and technical grade mirex at a few tenths percent. The mirex is dissolved in the soybean oil and then added to the corn cob grits. The corn cob grits are similar to coffee grounds in size and consistency, and when applied correctly, approximately 170 to 320 granules are deposited on each square meter of land (Markin, et al., 1972).

The common bait formulations originally used were designated 4X and 2X. The 4X bait contained 0.3 percent mirex, and was generally used for aerial application, at the rate of 1.4 kg/hectare (1.25 lb bait/ac). At

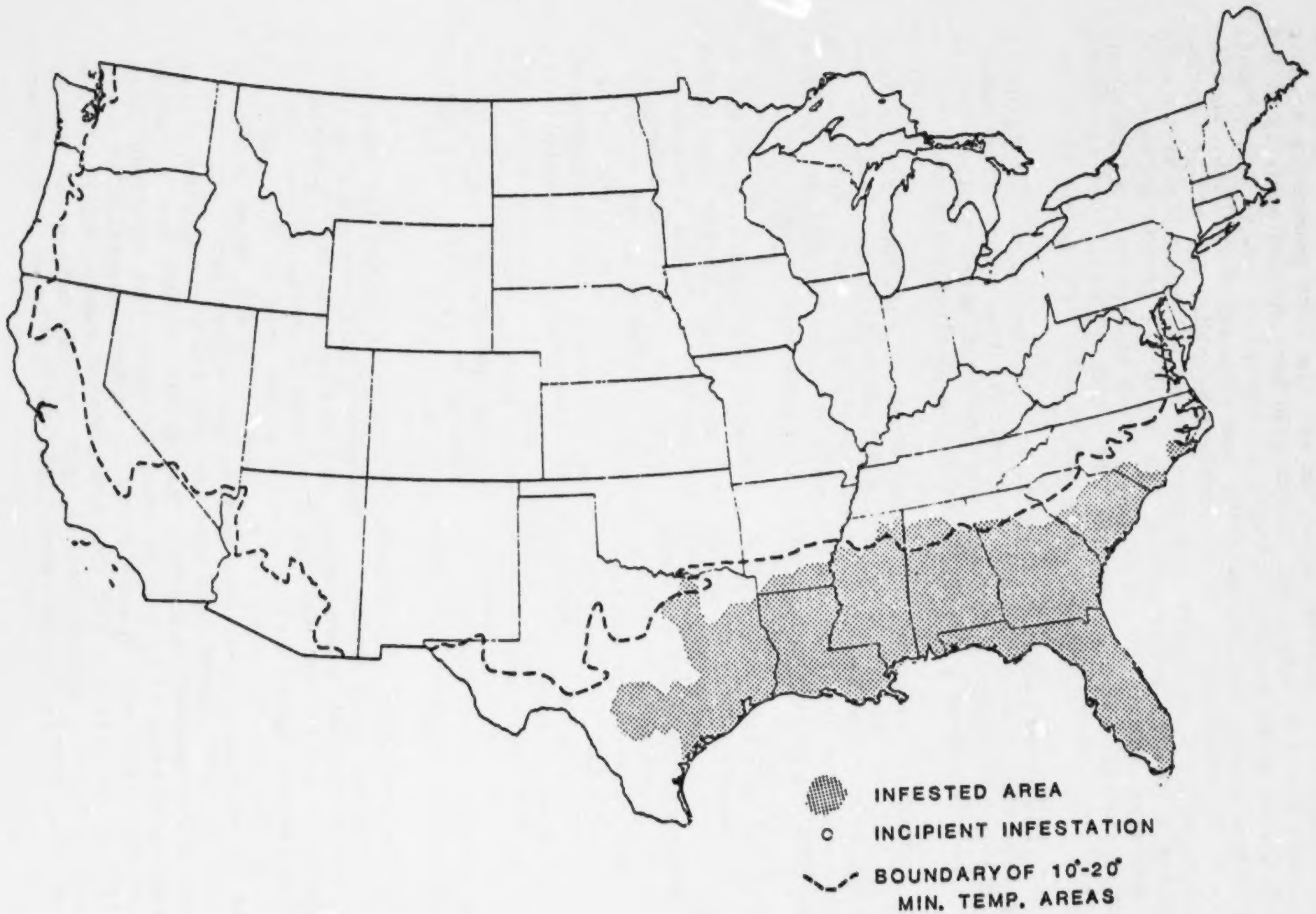
▨ INFESTED AREA
○ INCIPIENT INFESTATION
BOUNDARY OF 10°-20° MIN. TEMP. AREAS

Figure 2.6 Fire ant distribution, 1976.
Source: Council for Agricultural Science and Technology (1976).

this rate, actual mass of mirex applied was only 4.2 g/ha (1.7 g/ac). The 2X bait contained 0.15 percent mirex and was the form generally sold to home owners for individual use. It has also been applied aerially at 5.6 kg/ha (5 lb/ac) which deposits 8.4 g/ha (3.4 g/ac) of active ingredient. A new formulation, designated 10:5, has supplanted 2X bait. It also contains 0.15 percent mirex, but is prepared by adding 2/3 of the soybean oil, undosed, to the corn cob grits, and allowing the mixture to equilibrate a few days before adding the other 1/3 of the soybean oil, which contains all of the mirex. The theory is that by pre-loading the corn cob grits with plain soybean oil, the dosed oil will tend to remain on the surface; thus more of the mirex can be effective against the ants.

The 1977 program used a 0.1 percent bait at a rate of 2.5 kg/ha (1 lb/ac), equivalent to 0.45 g active ingredient per acre. Experimental degradable baits containing 0.05 and 0.025 percent mirex applied at 2.5 kg/ha (1 lb/acre) deposit only 0.23 and 0.11 g of mirex per acre.

In another variation of mirex bait formulation, the carrier is a material called "Furag", the partially carbonized corn cob residue remaining after corncobs are treated with sulfuric acid in the manufacture of furfural. Since the grits are somewhat smaller, and more friable, they can be utilized more effectively as bait (Brown, 1977).

When an infested area is treated with mirex bait, the bait is rapidly scavenged by the fire ant workers and placed in the mound. The toxicant is distributed throughout the colony, including the queen and brood, before any toxic effects become evident. Several weeks later, the ants die. This delayed toxicity is important because it allows sufficient time for distribution of the toxicant throughout the colony (Alley, 1973). In a few days the bait tends to become rancid, and less attractive to the ants. Also, since it leaves no effective residue, reinfestation into small cleared areas is quite rapid. Thus, its use by homeowners has been slow to gain acceptance; most homeowners preferred to use persistent pesticides, such as heptachlor, chlordane, or dieldrin (when these products were available) because they produced a rapid kill and left a residue to prevent reinfestation (Markin, et al., 1972).

After the successful 1961 field tests, programs against the imported fire ant using the newly developed mirex-corncob bait were begun in 1962. A Federal-State cooperative program was conducted by the U.S. Department of Agriculture until 1975. For the first several years, between 1962 to 1965, mirex (active ingredient) was applied at the rate of 8.4 g/ha (3.4 g/ac); from 1966 on, the rate was 4.2 g/ha (1.7 g/ac). Areas covered in 10 states and calculated mirex consumptions are summarized in Table 2.4 for FY 1962 to 1973. These data include only the mirex applied through the Federal-State cooperative program; they do not include areas treated by states independently, nor do they include the small-package over-the-counter sales made by many states to farmers, generally at cost, for individual application and spot treatment of mounds. While data are not available for the entire 1962 to 1975 period, data presented by Markin, et al., (1972) for 10 years (1962 to 1971) are illustrative.

TABLE 2.4. AGGREGATE AREAS TREATED WITH MIREX UNDER FEDERAL-STATE COOPERATIVE PROGRAM, FY 1962-1973[a]

	Areas Treated, Acres [b]									
	Alabama	Arkansas	Florida	Georgia	Louisiana	Mississippi	North Carolina	South Carolina	Tennessee	Texas	Total
					2.5 lb bait/acre (3.4 g mirex/acre)						
1962	46,370	21,790	1,340	477,310	263,440	79,050	7,000	37,540	0	40,260	974,100
1963	100,920	4,000	16,220	727,900	567,230	340,620	29,760	26,340	0	45,000	1,857,990
1964	78,110	25,290	90,440	970,450	441,750	232,030	144,590	324,120	0	40,910	2,347,690
1965	117,050	32,500	167,190	742,700	1,050,150	658,320	56,900	581,110	0	266,120	3,672,040
											8,851,820
					1.25 lb bait/acre (1.7 g mirex/acre)						
1966	294,210	61,910	961,160	2,039,780	1,335,510	963,800	232,660	36,430	0	203,090	6,128,550
1967	899,800	34,500	968,150	1,994,590	2,820,930	2,794,500	224,990	36,840	32,980	439,580	10,246,860
1968	326,580	20,290	3,388,700	5,530,900	439,900	1,650,810	77,650	1,353,900	0	44,460	12,833,190
1969	275,230	35,660	2,610,650	5,267,040	943,530	859,590	75,420	766,020	0	288,390	11,121,530
1970	285,590	162,450	204,830 [c]	8,557,390	1,642,220	515,150	60,320	2,951,540	0	124,030	14,508,520
1971	198,540	44,260	0 (c)	6,626,080	744,390	3,183,450	80	799,150	0	13,910 [c]	11,609,770
1972	354,050	0	99,460 [c]	5,615,680	1,100,000	3,326,160	1,580	565,810	0	2,380 [c]	11,065,120
1973	1,426,840	52,040	0 (c)	7,251,940	1,770,190	2,717,730	0	362,740	0	602,540 [c]	14,184,020
Subtotal											91,697,560
Total	4,403,290	494,690	8,508,140	45,801,760	13,119,240	17,321,210	910,950	7,841,540	32,980	2,115,670	100,549,380

Total mirex applied: 1962-65 30,095 kg (66,350 lb)
1966-73 155,885 kg (343,670 lb)
Total, 1962-73 185,980 (410,020 lb)

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[a] Source: U. S. Department of Agriculture (1977).

[b] Acre = 0.405 hectare.

[c] Extensive treatment performed under independent state program.

During this period, the records indicate that a total of 122,260 kg of mirex was applied, of which only 4,280 kg (3.5 percent) was credited to State-farmer programs, i.e., 96.5 percent of the total was applied under the Federal Cooperative Program. Since evidence from the infested southeastern states indicates that this ratio is also representative of the next several years, it seems certain that over-the-counter sales were a very minor segment of mirex consumption.

Two states, Florida and Texas, had significant state programs, independent of the Federal-State cooperative program. From 1970 on, Florida's participation in the U.S.D.A. program was impeded by application restrictions, including a requirement of minimum treatment blocks of 2,050 hectares (5,000 acres). Since assembly of blocks of this size posed problems in Florida, more reliance was placed on the Florida state program, which included both aerial and ground application. From 1970 to February, 1975, approximately 2,500 kg of mirex was applied under this program (Brown, 1977). Similarly, the state of Texas, over the past 4 to 5 years, has treated several million acres with mirex bait; this might account for several thousand additional kilograms of mirex (Ivie, 1977). Except for these two states, use of mirex for fire ant control by other southeastern states was quite minor.

In addition to the imported fire ant, several other species of locally important U.S. pests are effectively controlled by the use of mirex applied as bait formulations; these include the western harvester ant, Pogonomyrmex occidentalis, which infests millions of hectares of rangeland of the western states, and the Texas leaf-cutting ant, Atta texana, a serious pest of pine seedlings, hardwood trees and cereal and forage crops in some areas of east Texas and west central Louisiana. Mirex is one of the best control materials for the Texas leaf cutting ant, and is used commercially to protect timber areas, but it is a low volume usage (Ivie, 1977). No quantitative data on mirex usage for these other ant pests has been identified, but indications are that such use was also minor (Ivie, 1977). Mirex baits were also registered by Allied Chemical Company for yellow jacket control at one time, but no data on the magnitude of this use was found.

Beginning in 1970, mirex was also used in Hawaii to control populations of the big-headed ant (Pheidole megacephala) in pineapple fields (Bevenue, et al., 1975). This ant has a symbiotic relationship with the pineapple mealybug which transmits mealybug wilt, a serious pineapple disease. Control of the ant population keeps the disease in check (Johnson, et al., 1976). In 1972 about 34,500 kg (76,000 lb) of bait was used to treat 12,150 hectares (30,000 acres). Total active mirex was only about 100 kg. The use of mirex in Hawaii was temporarily suspended in 1972 by EPA, but the suspension was later stayed on condition that a one year monitoring program, approved by EPA, be conducted. In 1973, the stay was again continued, providing that a further mirex monitoring program would be carried out (Johnson, et al., 1976). These Hawaiian monitoring programs provided quantitative data on the fate of mirex in the environment; these data are described later (Section 7.2.2).

The total quantity of mirex represented by the 1962-73 Federal-State cooperative program summarized in Table 2.4 is approximately 186,000 kg (410,000 lb). During the same period, the sales of mirex by Hooker Chemicals and Plastics Corporation (Table 2.5) totalled approximately 345,000 kg (760,000 lb). As shown above, neither the State-farmer programs nor independent State fire ant control programs account for the difference of 159,000 kg, nor is there evidence that significant quantities were used for the control of other U.S. pests. Thus, it is concluded that the Federal-State cooperative program accounts for most (probably over 90 percent) of the mirex used as a pesticide in the U.S. It is believed that most of the difference between the amounts of mirex sold and the amounts applied can be attributed to export sales. Markin, et al., (1972) noted that an unknown amount is produced and shipped abroad for the control of other ants, particularly the leaf cutter ant in South America. In addition, it may be used in Brazil for control of the native fire ant. For example, Hooker records show that one-fourth (approximately 4,600 kg) of 1975 mirex sales was destined for Brazil. Further, 40,600 kg of the total stock of 174,200 kg remaining on hand as of May, 1976, was on consignment to Brazil (Hooker Chemicals and Plastics Corporation, 1976).

The registration for 4X mirex bait was cancelled, effective December 1, 1976, and use of existing stocks permitted only through December 31, 1976. The registration for 10:5 bait was cancelled, effective December 1, 1977; aerial application of existing stocks was permitted through December 31, 1977. Bait existing December 1, 1977 could be packaged and sold in 5-lb bags for ground application until June 30, 1978. The cancellations also specified that no more than 27,200 kg (60,000 lb) of technical mirex could be used for the two baits during this phase-down period. (U.S. Environmental Protection Agency, 1976b).

Overall, it appears clear that the total quantity of mirex applied for control of the fire ant in the U.S. through 1975 was something under 400,000 kg, probably about 250,000 kg. Usage during the phase down period may increase total usage to 275,000 kg.

2.5.2 Kepone

2.5.2.1 Manufacture--

Kepone was developed by the Allied Chemical Corporation in the early 1950's; original patents were granted in 1952 (Kelly, 1976a). As indicated in Section 2.2, the commercial manufacturing process was based on reacting hexachlorocyclopentadiene with SO_3, with subsequent hydrolysis to form Kepone.

The procedure used for the early manufacture of Kepone at the Allied Chemical Corporation semiworks plant at Hopewell, Virginia, is described in an Allied Research Informal Memorandum appended to an EPA report attempting to estimate Kepone discharges from the Allied plant (Smith, 1976). In this preparation procedure, 3442 kg (7,590 lbs) of hex was charged to a reactor, to which was added 11.3 kg (25 lbs) of antimony chloride catalyst, a small quantity of water (700 g), and 1,513 kg (3,336 lb) of sulfur trioxide (50 percent excess). The mixture was heated to 85 to 90 C for 10 to 12 hours. The reaction mass was quenched in 6 percent aqueous sodium hydroxide solution to hydrolyze it and put it into solution. The solution was neutralized with sulfuric acid to a pH of 5 to 6 to precipitate the Kepone. After digestion and cooling, the Kepone was centrifuged and washed with warm water to remove sodium sulfate, sodium chloride, and excess sulfuric acid, and was then dried. The average yield of the finished product was 2,517 kg (5,500 lb), containing 5.2 percent water. On the basis of hex charged, yield was 81 percent. Washings were discharged to the sewer. On the basis of a Kepone solubility of $\sim$ 2 ppm and the volumes of aqueous discharges, they were calculated to contain from 40 to 80 g of soluble Kepone per day of operation (Smith, 1976).

No discussion of the fate of the approximately 20 percent of hex not forming Kepone has been found in the literature. Since the solubility of hex is, like that of Kepone, quite low (0.81 ppm according to Lu, et al., 1975), the soluble losses of unreacted hex should have been negligible. However, under the reaction conditions employed, attack by the SO_3 could well have resulted in sufonation and sulfation of the hex to form various water-soluble by-products.

For a number of years after its development, Kepone production was limited, with Allied and two subcontractors manufacturing it only sporadically. On the basis of receipt records at the Allied Chemical's formulating and shipping plant at Baltimore, Maryland, technical Kepone was initially received in 1951 from Allied Chemical's development laboratories (1,360 kg). No more was received until 1959, when the Nease Chemical Company, State College, Pennsylvania, supplied 5,680 kg (12,530 lb). Nease Chemical also supplied small amounts in 1962 and 1963, and the Hooker Chemicals and Plastics Corporation, Niagara Falls, New York, was a supplier in 1965. (Allied also accepted 6,230 kg of residual inventory of off-specification material from Hooker in 1967 (Ferguson, 1975).

Demand began to grow in the late 1960's, as banana pests started to develop immunity to some widely used pesticides, and Allied itself took over production at its semiworks plant located at the Hopewell, Virginia, chemical complex (Bray 1975). Allied Chemical reportedly produced from 680 to 1,130 kg/day (1,500 to 2,500 lbs/day) intermittently at the semiworks, during the period from 1966 to 1974 (Kelly, 1976b). Kepone was produced only as needed, and production runs seldom lasted more than 90 days at a time (Bray, 1975). Increasing demand for the pesticide, particularly from Europe, and a need for the semiworks facility for the manufacture of other chemicals evidently led to the transfer of manufacture to the Life Sciences Products Company (LSPC) of Hopewell, Virginia, beginning in early 1974 (Kelly, 1976b; Jaeger, 1976). Production continued to increase, exceeding 385,000 kg (840,000 lb) in 1974. During the first six months of 1975 preceding the closing of the LSPC plant over 384,000 kg (846,000 lb) were produced. Total receipts of technical Kepone at Allied's Baltimore formulating plant, as compiled by Ferguson (1975), are shown in Table 2.5.

Total production, using Ferguson's compilation, was nearly 1,600,000 kg (3,500,000 lb). Very little of this was used in the United States. One EPA estimate (U.S. Environmental Protection Agency, 1975b) was that 99.2 percent of annual production was exported to Latin America, Africa, and Europe. Another report (Sterrett and Boss, 1977) states that over 90 percent of the nearly 770,000 kg (1,700,000 lb) manufactured by LSPC was exported to these same areas for control of insect pests.

2.4.2.2 Use--

There are no large scale agricultural uses for Kepone in the U.S.; most of the moderate to small annual U.S. consumption appears to have been for domestic roach and ant control. Most of the Kepone produced was shipped overseas to a West German firm for conversion into other pesticide products (Kelly, 1976b).

One of Kepone's principal uses is for control of the banana root borer. A 5 percent dust is registered for use in Puerto Rico on banana plants. Directions call for surface application of 3.6 kg/ha (8 lb/ac) of active ingredient, and allow for application at six month intervals (U.S. Environmental Protection Agency, 1976c). In an earlier document (U.S. Environmental Protection Agency, 1975b), the dosage was given as 2.2 kg/ha (2 lb/ac) or 2.8 g (0.1 oz) per plant.

This constitutes the only known use of Kepone on food crops.* On non-food crops, Kepone has been used for control of wireworms in tobacco fields, and in bait and traps to control ants and roaches in indoor and outdoor areas. (U.S. Environmental Protection Agency, 1975b). According to the notices of Rebuttable Presumption Against Registration and Continued Registration (U.S. Environmental Protection Agency, 1976d,e) there were at that time 27 companies manufacturing roach or ant traps or baits. Active ingredients were limited to 0.125 percent Kepone.

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*Revocation of the tolerance, or maximum residue level, of 0.01 ppm in or on bananas has been promulgated by EPA (U.S. Environmental Protection Agency, 1978) at the request of the only registrant. This will essentially eliminate its use on bananas.

TABLE 2.5 RECEIPTS OF TECHNICAL KEPONE AT BALTIMORE, MARYLAND FORMULATING PLANT OF ALLIED CHEMICAL CORPORATION[a]

Year	Quantity kg	(lb)	Source
1951	1,360	(3000)	Allied Development Laboratory
1952-58	None Reported		
1959	5,710	(12,530)	Nease Chemical Company
1960	4,990	(11,000)	Nease Chemical Company
1961	None Reported		
1962	1,430	(3,150)	B&A Works
1963	18,125	(39,960)	Nease Chemical Company
	5,550	(12,245)	B&A Works
1964	None Reported		
1965	16,270	(35,880)	Hooker Chemical and Plastics Corp.
1966	1,035	(2,285)	Nease Chemical Company
	35,435	(78,125)	Allied/Hopewell
1967	6,260	(13,800)[b]	Hooker Chemical and Plastics Corp.
	47,990	(105,800)	Allied/Hopewell
1968	36,535	(80,550)	
1969	46,990	(103,600)	
1970	41,460	(91,400)	
1971	204,800	(451,515)	
1972	176,970	(390,150)	
1973	100,435	(221,425)	
1974	72,260	(159,300)	
	385,370	(849,600)	Life Science Products Company
1975	384,020	(846,625)[c]	Life Science Products Company
Total	1,592,895	(3,511,940)	

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[a] Source: Ferguson (1975).

[b] Off-specification residual Hooker inventory.

[c] Through July, 1975.

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3.0 EFFECTS ON MICROORGANISMS

3.1 SUMMARY

Laboratory investigations have shown that mirex in concentrations up to 100 ppm did not inhibit hydrolysis of gelatin, starch, lipid, casein or affect other metabolic functions of estuarine microorganisms. Concentrations up to 1000 ppm of mirex did not significantly alter either microbial populations or microbial activities in estuarine sediments. Various degradation products of mirex, notably Kepone and reduced Kepone may, however, be more toxic to microorganisms than the parent compounds, because of their higher water solubility.

Mirex concentrations up to 20,000 ppm (1 gram mirex to 50 g soil) had no effect on total populations of soil bacteria or fungi, but actinomycete populations were reduced at 10,000 ppm in some soil types. Degradation of mirex by soil microorganisms has not been observed in either aerobic or anaerobic conditions. Sewage sludge microorganisms, on the other hand, appear to be capable of metabolizing mirex under anaerobic conditions, but the process is very slow.

Kepone applications of 22.0 kg/ha (19.6 lb/acre) to three soil types in the Nile Delta altered fungi, actinomycete and bacteria populations. Disagreement exists concerning the reasons for population resurgence of the microorganisms and the decline of the biological activity of Kepone. Ammonification of peptone, decrease of nitrification of ammonium sulfate, temporary accumulation of nitrites, and decrease of nitrates have been observed in soils treated with Kepone. Elevated nitrite levels may be toxic to some plant species.

Beneficial bacteria were reported kiled in sewage treatment facilities at Hopewell, Virginia, upon the addition of Kepone wastes.

3.2 ESTUARINE MICROORGANISMS

Brown, et al., (1975) conducted a series of related experiments to establish the chemical and microbiological fate of mirex in the estuarine environment and to determine its effects on important estuarine microorganisms and their activities. The most important results of this study were: (1) At least 95 percent of mirex in aqueous solution is removed by adsorption by organic matter (dead bacterial cells), kaolinite clay and montmorillonite clay within 2, 7, and 30 days, respectively. (2) No significant decrease in mirex concentration occurred in a sediment-water system exposed to sunlight for 130 days, but some evidence of disappearance was noted in purely aqueous solutions. (3) Results from disc assay tests of bacterial growth inhibition were inconsistent from batch to batch of technical grade mirex, with some batches producing little or no growth inhibition of the bacterial isolates while others showed marked inhibition. Gas chromatographic studies demonstrated the presence of varying amounts of unidentified impurities in different batches of mirex which may account for such discrepant findings. (4) In culture studies, mirex at concentrations up to 100 ppm did not inhibit hydrolysis of gelatin, starch, lipid, casein and chitin, glucose fermentation, H_2S production, nitrate reduction, ammonification and methane utilization. The same concentrations also did not inhibit methane utilization and nitrification by estuarine sediments. Ten ppm of mirex did not inhibit the metabolic activity of estuarine pond water organisms but did inhibit primary productivity. (5) Concentrations of 1000 ppm mirex did not affect the growth curves of bacterial cultures in broth. (6) Microcosm studies confirmed the lack of inhibition by mirex. Concentrations of 1000 ppm did not significantly alter the mixed microbial populations or the microbial activities of estuarine sediments. (7) Degradation products of mirex, especially Kepone and reduced Kepone, were shown to be far more toxic than the parent compounds. (8) Ultraviolet irradiation of moist mirex resulted in a bacteriologically toxic material.

The only appreciable microbiological activity affected by mirex at concentrations below 100 ppm in laboratory experiments was the inhibition of primary productivity. In the estuarine environment mirex would not be expected to have any significant effect on primary productivity because the majority of phytoplankton are in the aqueous phase, whereas mirex tends to become associated with the sediments. Of critical importance was the finding that purified mirex was not toxic to microorganisms while some batches of technical mirex and UV-irradiated mirex were toxic.

Several degradation products of mirex were tested against a number of bacterial cultures using a disc assay technique. Neither the monohydrogen derivative or the dihydrogen derivatives with substitutions at the 2 and/or 2, 8 positions inhibited any of the 10 cultures tested, whereas degradation products with substitution at the 5 and/or 10 positions were highly toxic. It was not known if any of these compounds were present in the mirex employed in the investigation.

Brown, et al., concluded that other studies on the toxicity of mirex should be re-evaluated. This is especially true because one of the breakdown compounds studied in this investigation has the same retention times as the mirex itself and because the concentrations of degradation products that would cause observable toxicity would not be detected by normal gas chromatographic procedures. All of these compounds are more polar than mirex and should therefore have higher water solubility than mirex. These facts may be important for toxicity studies of mirex in water.

3.3 SOIL MICROORGANISMS

3.3.1 Mirex

Very little information on the behavior of mirex in soils is available. One study conducted by Jones and Hodges (1974) examined the persistence of mirex in soils and the effects of mirex on soil microorganisms. Their study showed that up to 1 gram of mirex per 50 g of soil (20,000 ppm) had no demonstrable effects on total populations of soil fungi or bacteria. However, concentrations of 0.5 and 1.0 g significantly reduced populations of actinomycetes in one out of three soils. A variety of soil fungi and a few bacteria colonized mirex bait but failed to degrade the mirex. No degradation of mirex occurred in nine aerobic soils or four anaerobic lake sediments after 6 months incubation or in liquid cultures of bacteria after 1 month. ^{14}C-labeled mirex in bait placed outdoors for 6 months remained primarily in the bait particles; only 6.6 percent was found in the top 15 mm of soil. No mirex leached through a 100 x 110 x 45 mm soil layer with approximately 3 liters of collected rainfall.

These field studies support the conclusion drawn from laboratory tests that mirex is not degraded by soil microorganisms. Under field conditions, mirex probably remains in unconsumed bait on the soil surface until the bait is completely decomposed. Decomposed bait and mirex are probably incorporated into the soil slowly. Because of the small amount of mirex applied to the soil (1.7 g/acre application rate) and the low solubility of mirex it is doubtful that significant amounts leach into groundwater following mirex application for fire ant control.

3.3.2 Kepone

Virtually the only available reports on the effects of Kepone on plants, soil microorganisms and transport and distribution of Kepone in soil are those by Gawaad, et al., (1971, 1973a, 1973b, and 1975). Information used in this report comes from abstracts and secondary sources because the original reports by Gawaad, et al., were written in Hungarian. Unfortunately, translations were not available at the time of writing.

Gawaad, et al., (1971) investigated the quantities of Kepone and other chlorinated hydrocarbon and organophosphate pesticides leached from several different types of soil. Simple percolation tests were conducted using soil-filled metal cylinders which had been perforated at the bottom to allow collection of the leachate. After treatment with the insecticide, the water draining from each cylinder was tested for insecticidal concentration by bioassay, using Daphnia. Kepone applications at a rate of 22.0 kg/ha (19.6 lb/acre) to three soil-types in the Nile delta altered fungal, actinomycete and bacterial population levels for as long as 45 days (when compared with controls). The significance of this effect from an agronomic standpoint is unknown especially in view of the unrealistic application rates used. The magnitude and duration of the effect on population levels differed with soil type. The microbial populations appear to return to normal levels after 15 days despite pronounced fluctuations. Carbon dioxide production, an indicator of the activity of microorganisms, followed nearly the same pattern. The authors attribute the strong increase in both the count and the activity of the soil fungi after the first 7 days to the intense breakdown and detoxification of the pesticides by the microorganisms (Gawaad, et al., 1973a). The U.S. Environmental Protection Agency (1975) criticized these conclusions, and suggested a number of more plausible explanations which could account for such findings. Suggested alternative explanations were:

(1) The return to a normal population was a result of proliferation of organisms tolerant to the pesticide.

(2) The biological effects of Kepone did not persist in the soil due to some physical or chemical reason such as leaching, chemical hydrolysis or volatilization.

In another similar report, Gawaad, et al., (1973b) studied nitrogen transformations in soils treated with Kepone. During the first 2 weeks following soil treatment, ammonification of peptone and nitrification of ammonium sulfate decreased. Although nitrites accumulated in the treated soils, the nitrate level decreased during the first 15 days, indicating that organisms of the genus *Nitrobacter* had been suppressed whereas organisms of the genus *Nitrosomonas* had been suppressed to a lesser degree or not at all.

3.4 SLUDGE MICROORGANISMS

3.4.1 *Mirex*

Recently, studies by Andrade, et al., (1975) have indicated that mirex is metabolized very slowly by sewage sludge organisms under anaerobic conditions. The single metabolite generated has been tentatively identified as the 10-monohydro product (see Section 2.3.1, Structure II). After 75 days incubation, autoradiograms of the thin-layer plates showed two distinct spots for the anaerobic samples, but only one spot for the aerobic samples and the controls. The ratio of the counts of the mirex zone was calculated for each sample as quantitative evidence of the presence of a small amount of a metabolic product. This data is shown in Table 3.1.

The data indicate that under the experimental conditions used sewage sludge microbes degrade mirex under anaerobic conditions. Evidence provided by the controls as well as the fact that the incubation was done in the dark eliminated the possibility that the non-mirex spot seen could have been caused by chemical degradation or photolysis. The data from the mirex stock solution and controls eliminate the possibility that the metabolite resulted from autoradiolysis.

TABLE 3.1. RATIO OF RADIOACTIVITY IN MIREX AND METABOLITE ZONES ON THIN LAYER SILICA GEL PLATES[a,b]

Sample	Ratio Mirex/Metabolite
Anerobic sludge	88
Anaerobic control	761
Aerobic sludge	636
Aerobic control	470
Mirex stock solution	440

[a] Source: Andrade and Wheeler (1974). Reprinted, with permission from Bull. Environmental Contamination and Toxicology. (c) Springer-Verlag New York Inc. (1974).

[b] These experiments were replicated three times using different samples of sludge. The data presented here are from one experiment and are representative of the three replications.

3.4.2 Kepone

Few studies have been published concerning the topic of Kepone metabolism in sewage and sludge. Presumably, Kepone is highly toxic to sewage digesting microorganisms. Kepone was reported to have killed massive amounts of beneficial bacteria in the vital digester system at the sewage treatment facility in Hopewell, Virginia, after Kepone wastes were dumped into the city's sewage system by Life Sciences Products Company (Bray, 1975). The exact nature of the discharge and the amount of Kepone discharged in connection with this incident have not been reported.

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4.0 EFFECTS ON PLANTS

4.1 SUMMARY

Little work has been done on the effects of mirex and Kepone on field and pasture crops. Johnson grass and annual rye grass showed initial significant reduction in growth rate at 0.15 ppm mirex; tall fescue and alfalfa at 0.3 ppm and alsike clover at 0.70 ppm. One study indicated that uptake and accumulation of mirex was directly related to the mirex concentration of the rooting medium. Soybean, garden bean, sorghum and wheat seedlings showed a similar uptake preference. Plant root preparations known to metabolize other chlorinated hydrocarbons failed to yield any metabolite when incubated with mirex. Information on Kepone metabolism by plants was not available.

4.2 FIELD AND PASTURE CROPS

Examination of all types of biological samples for mirex residues has generally revealed that the smallest amounts appear to be found in plants (Markin, et al., 1972). Earlier work by Allied Chemical Corporation indicated that no mirex residues could be found in a large selection of field and vegetable crops (Mirex Advisory Committee, 1972). There is some evidence that extremely low levels of mirex may be translocated from the soil into growing plants such as bahiagrass roots and foliage. Mehendale, et al., (1972) also indicated the possibility of uptake by plants of mirex dissolved in water. Certain aquatic algae were found to contain relatively high concentrations of mirex (Markin, et al., 1972). Data on aquatic plants are included in a subsequent section on aquatic biota.

Little work has been done on the effects of mirex and Kepone on plants; the areas of seed germination, seedling emergence and survival and early growth of seedlings have been especially neglected. A study reported by Rajanna and de La Cruz (1975) investigated the phytotoxic effects of mirex on several commonly grown field and pasture crops: crimson clover, Johnson grass, annual rye grass, tall fescue, alsike clover, and alfalfa. Mirex concentrations employed in the experiment ranged from 0.3 to 3.5 ppm. Germination experiments utilized germination blotters presoaked in the prepared mirex solutions. Percent germination based on number of seeds planted was calculated daily for 21 days. Emergence and seedling growth were calculated for four replicates of 100 seeds each.

The results of the experiments indicated a significant reduction in both germination and emergence as the concentration of mirex increased. Visual examination of the seedlings revealed poor development, suggesting that uptake of mirex by the young seedlings caused the damage. Dry weight of seedlings decreased at higher mirex concentrations. Crimson clover, Johnson grass and annual rye grass showed initial significant reduction in growth rate at 0.15 ppm mirex; tall fescue and alfalfa at 0.30 ppm concentration, and alsike clover at 0.70 ppm. The exact mode of toxicity of mirex on germination and development of seedlings is not well understood; however, these studies did not show that uptake and accumulation of mirex was directly related to the mirex concentration of the rooting medium. The authors suggest that the phytotoxic effects observed could be due to a rapid diffusion of water containing mirex during the germination process.

In a related study, de La Cruz and Rajanna (1975) found that soybean, garden bean, sorghum and wheat seedlings take up and accumulate varying amounts of mirex residue when grown in substrates containing from 0.3 to 3.5 ppm mirex. Varying amounts of mirex residues were taken up by the different parts of the plants grown either in soil or sand, as shown in Table 4.1. In general, the roots have the highest quantity of mirex residue. Regardless of difference in species, all plants grown in loamy sand generally accumulated higher amounts of mirex than those grown in field soil. Although the concentrations of mirex used in the experiment are higher than those found in nature, mirex uptake and accumulation by crop seedlings was demonstrated. At present, there is little available information on the biochemical fate of mirex once it enters the plant body or the persistence of mirex in plant tissues beyond the 4-week duration of this study.

Little information is available concerning the effects of Kepone on plants, However, Kepone was included in a study on the effects of soil insecticides on germination of cotton seeds, growth, dry weight and qualtiy and quantity of cotton yield. Ironically, test results indicated that yield quality was favorably affected due to a higher lint percentage. Insecticide residues in the seed were less than 1 ppm under several different rates of application (Gawaad, et al., 1975).

4.3 METABOLISM

Plant root preparations known to metabolize other chlorinated hydrocarbons were incubated with mirex but failed to yield any metabolite (Mehendale, et al., 1972). In the plant study, 2-week-old pea and bean plants were grown for 48 hours in water containing 1 to 19 ppm of ^{14}C-mirex (introduced into 1 percent methylcellosolve solution). Various parts of the plants were then analyzed for radiocarbon. Mirex was absorbed by both bean and pea roots in proportion to the concentration of ^{14}C-mirex in water as shown in Table 4.2. Translocation to the aerial parts of 7 to 8 percent of the mirex in the roots occurred, indicating the possibility that plants do take up mirex in spite of its low water solubility. The authors failed to comment on the nature of the material in the roots or shoots. Incubations with preparation from these species for up to 24 hours gave no evidence of metabolite formation from mirex.

Information on Kepone metabolism by plants was not available. However, the insecticide Kelevan, which degrades to Kepone, has been investigated. Sandrock, et al., (1974) examined the metabolites and balance in potatoes and soils of ^{14}C-Kelevan. Eleven weeks after application of ^{14}C-labeled Kelevan (ethyl Kepone-5-levulinate) to soils the main degradation product was Kelevanic acid and by-products were Kepone and Keponeacetic acid. In another experiment, 5.4 mg ^{14}C-labeled Kelevan was sprayed on potato leaves. After harvesting, the ^{14}C compounds were found in total potato plants, soil, and drainage water in the following amounts:

In potato plants:	0.03 mg	Kelevan
	0.03 mg	Kepone
	0.24 mg	Kelevanic acid
	0.04 mg	Keponeacetic acid
	0.02 mg	Unknown extractable compounds
	0.01 mg	Unknown extractable compounds
Total in plant =	0.37 mg	^{14}C compounds.
In soil:	0.08 mg	Kelevan
	0.06 mg	Kepone
	0.96 mg	Kelevanic acid
	0.18 mg	Keponeacetic acid
	0.06 mg	Unknown extractable compounds
	0.08 mg	Unknown extractable compounds
Total in soil =	1.42 mg	^{14}C compounds.

Total in drainage water, 0.05 mg ^{14}C compounds.

The ^{14}C balance in this study was 6.9 percent in potato plants, 26.3 percent in soil, 0.9 percent in drainage water, and 65.9 percent loss to the air.

TABLE 4.1 CONCENTRATIONS OF MIREX RESIDUES IN DIFFERENT PARTS OF 4-WEEK-OLD CROP SEEDLINGS GROWN IN LOAMY SAND AND EXPERIMENTAL FIELD SOIL[a]

	Concentration, ppm, of mirex in the experimental loamy sand[b]			Concentration, ppm, of mirex in the experimental field soil[b]		
Plant parts	3.4	0.8	0.31	3.5	0.8	0.3
Garden beans						
Growing tip	0.27	0.09	0.04	0.12	0.06	0.02
Leaves	0.40	0.19	0.01	0.20	0.11	0.01
Upper half stem	0.31	0.28	0.17	0.31	0.22	0.10
Lower half stem	0.79	0.38	0.20	0.63	0.25	0.02
Root	1.68	0.69	0.23	1.18	0.49	0.21
Soybean						
Growing tip	0.31	0.08	0.05	0.09	0.06	0.01
Leaves	0.36	0.18	0.03	0.21	0.12	0.10
Upper half stem	0.73	0.29	0.13	0.35	0.18	0.11
Lower half stem	0.92	0.31	0.27	0.76	0.27	0.12
Root	1.47	0.49	0.32	1.25	0.49	0.17
Sorghum						
Leaves	0.40	0.33	0.18	0.22	0.20	0.11
Stem	1.60	0.46	0.21	0.51	0.31	0.17
Root	1.71	0.67	0.28	0.81	0.44	0.20
Wheat						
Leaves	0.21	0.19	0.04	0.17	0.18	0.09
Stem	0.95	0.32	0.21	0.56	0.20	0.20
Root	1.33	0.55	0.36	1.17	0.27	0.23

[a] Source: delaCruz and Rajanna (1975). Reprinted, with permission, from Bull. Environmental Contamination and Toxicology. (c) Springer-Verlag New York, Inc (1975).

[b] Analysis by GC.

TABLE 4.2 UPTAKE OF MIREX BY PEA AND BEAN PLANTS[a,b]

Mirex-^{14}C in water ppm	Peas, ppm		Beans, ppm	
	Roots	Shoots	Roots	Shoots
1	6.487	0.586	8.478	0.496
5	40.696	1.521	45.364	3.867
10	71.285	8.408	101.604	6.834

[a] Source: Mehendale, et al. (1972). Reprinted, with permission from Bull. Environmental Contamination and Toxicology. (c) Springer-Verlag New York, Inc. (1972).

[b] Mirex-^{14}C (5×10^6 dpm) along with desired amount of unlabeled mirex was introduced in water as methylcellosolve (1 percent) solution.

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5.0 EFFECTS ON BIOTA

5.1 SUMMARY

The ecological significance of mirex in the aquatic environment does not necessarily depend upon the amount of the residue that enters the water. Instead, the ecological and biological significance of mirex depends upon its mode of incorporation into aquatic organisms and its effects on organisms chronically exposed to low concentrations of this pesticide. The solubility of mirex in fresh water does not exceed 1 ppb (0.001 ppm), and may be 0.2 ppb or less in seawater (Section 2.3.1.1). Rather than remaining suspended in the water column for any extended length of time, mirex is rapidly adsorbed onto various organic particles and removed to the sediments where it may have an appreciable lifetime.

Mirex, in environmental concentrations, does not appear to be directly toxic to marine algae or freshwater phytoplankton in terms of population growth, oxygen evolution, or primary productivity. Several species of marine algae tend to accumulate (but not metabolize) mirex from water. Therefore, these organisms may very well act as physical agents in transporting mirex into estuarine or marine ecosystems and to consumers in the food chain.

Bioaccumulation and biomagnification potential of mirex vary among invertebrate species and among the life stages of certain of those species. Bioaccumulation is generally a function of exposure period and mirex concentration. The mirex levels accumulated by invertebrate species suggest the potential hazard involved if these organisms transport the pesticide to secondary and tertiary consumers in aquatic food chains or to human consumers of these food commodities.

Mirex is accumulated by fish from water and from food chain organisms. It accumulates mainly in the fatty tissue and visceral organs of most fish species. However, some species such as bass, bream, catfish, and mullet have been found to contain at least 1 ppm of mirex in their edible portions. Mirex has, for the most part, relatively insignificant effects on fish growth and oxygen consumption. Some pathological developments such as granulomatous kidney lesions and edematous gill changes were noted among mirex-fed goldfish. Mortalities among fish species were not always found to be mirex-related.

Laboratory and field studies show that the food chain is the mode of transport of mirex in natural populations. Mirex is concentrated by algae, invertebrates, and fish. The total environmental significance of mirex residues in nontarget species, however, is mostly unknown. Levels well over 1 ppm are found in a number of animals at the top of the food chains; some of these fish and invertebrate species are commonly used for human consumption. Therefore, man does have direct access to a potentially hazardous chemical through his food chain.

Effects of mirex on invertebrate species, especially estuarine crustacens, vary from irritability and loss of equilibrium to death. Juvenile brown and pink shrimp and juvenile blue crabs were found to be the estuarine species most susceptible to mirex. Of particular interest is the delayed mortality exhibited by invertebrate species (e.g., crayfish, juvenile pink shrimp, and juvenile blue crabs) exposed directly or indirectly to mirex

Kepone, like mirex, has a low solubility in freshwater and seawater. The ecological and biological significance of Kepone in aquatic systems depend upon its bioaccumulation by aquatic organisms and its transfer potential among organisms comprising aquatic and human food chains. Algal species accumulate Kepone from water and may pass the pesticide along the food chain to man. The few data available on the effects of Kepone show that Kepone reduces algal growth. Exposures to Kepone concentrations ranging from 0.35 to 1.0 ppm are deemed to be sufficient to disturb or destroy the lowest level of the aquatic food chain, thereby directly affecting productivity at other levels of the food chain.

Although much of the data on Kepone acumulation by invertebrates are based on laboratory exposures to unrealistically high concentrations, these experiments show that invertebrates are capable of bioaccumulating Kepone to dangerously high levels. Kepone bioconcentration factors vary among invertebrate species because of the difference between depuration rates. Kepone is acutely and chronically toxic to estuarine inverterate species. Besides mortality, effects of Kepone include loss of equilibrium, reduction of reproductive success, and decrease in shell growth.

Kepone is bioaccumulated and persistent in fish species such as sheepshead minnows and spot. Even juvenile progeny of adult fish (e.g., sheepshead minnows) exposed to Kepone, hatched and grown in Kepone-free water, contained Kepone. One reason is that fish have slow depuration rates as compared with some invertebrates such as oysters. Female fish sometimes have Kepone residues higher than those of male. One of the largest Kepone reserves was in the edible portion of the contaminated fish. Symptoms of Kepone poisoning in fish have been correlated with concentration and duration of exposure. Among several fish species exposed to Kepone under varying laboratory conditions, symptoms ranged from diminished activity and emaciation to abnormal development and mortality. Yet another observed effect of Kepone is ATPase inhibition which reduces the energy available to the organism.

The accumuation, transfer, and loss of Kepone has been verified in estuarine food chains via laboratory bioassays. Transfer and retention of Kepone from algae by invertebrates with high depuration rates (e.g., oysters) are inefficient, while transfers between invertebrates and between invertebrates and fish are efficient. Because of the efficient transfer of Kepone between fish food organisms and fish, there is a great potential for Kepone transfer to humans via consumption of fish.

Mirex does not appear to present a serious acute toxicity problem to nontarget terrestrial invertebrates following the proper usage of mirex bait. Mirex residue levels are low, typically 0.1 ppm in most phytophagous animals collected from mirex treated areas. However, general scavengers such as crickets and cockroaches, which may consume the bait pellets directly, have been shown to acumulate mirex residues in the 10 to 30 ppm range. Information regarding Kepone toxicity in these species comes only from experimental situations, but orchard monitoring studies suggest that Kepone, too, is not acutely toxic to predaceous insects at up to five times the typical dosage used in agricultural applications.

Information on amphibian and reptile responses to both pesticides is inadequate, however, environmental surveys have consistently found mirex residues to be lower in these species than in birds and mammals.

In comparison with mammals and fish, birds are less sensitive to mirex in either acute or chronic exposures. Chickens, for example, require a daily consumption of 300 to 600 ppm mirex in food to produce reproductive effects; subacute toxicity tests on a variety of bird species produced mortality at feeding levels of 200 to 500 ppm after 30 to 111 days. Mirex is remarkably accumulative in tissues following long-term dietary exposures. The highest residues are found in skin and fat. These tissues can accumulate over 200 times the concentration of mirex in the diet. One of the most interesting characteristics in birds is the differential residue patterns seen between males and females. Because mirex accumulates in egg yolks, egg laying represents an important excretion route. Long-term metabolic studies of quail, for example, have shown that hens establish an equilibrium between mirex intake and elimination through the eggs, while in male residues continue to accumulate and show little tendency to reach a plateau. Reproductive effects such as decreased egg production, decreased survival of chicks, reduced hatchability and eggshell abnormalities have been observed in several bird species. The threshold dosage, required to elicit reproductive effects (assuming generalization across avian species) probably lies in the range of 40 to 100 ppm mirex in the diet.

Wildlife sampling surveys in or near treated areas have shown that birds as a group contain relatively high levels of mirex. These high levels presumably relate to the fact that birds consume a great deal more food on a per weight basis than do insects, reptiles and mammals. The most important determinants of mirex levels in these birds are dietary patterns (i.e., carnivorous versus herbivorous) and whether or not the home range extends beyond the treated areas. Highly lipid-soluble chemicals such as mirex are bioconcentrated by fish which, in turn, provide potent sources of mirex to predatory birds as seen in herring gulls in the Great Lakes region (See Section 5.3.3.5).

Like mirex, Kepone is not very toxic to birds as an acute poison. In subacute dosages, Kepone is considerably more toxic than mirex. Tremors, pathologic changes in the liver and reproductive difficulties similar to an "estrogenic effect" are seen in both sexes at dietary levels of 200 ppm of Kepone. Furthermore, the finding of reduced survival in stress situations (due to mobilization of Kepone-laden fat stores) suggest that a large body burden of Kepone, while not producing symptoms of toxicity in normal situations, may dramatically reduct the ability of the birds to successfully cope with environmental stresses.

Mirex administered as an acute oral dose is not very toxic to those mammalian species that have been tested. Acute LD_{50}'s for rats lies in the range of 365 to 740 mg/kg; many other organochlorine and organophosphorus pesticides have much lower LD_{50}'s. The most commonly observed subacute toxic effects of mirex in mammals are weight loss, increased liver weight and reproductive difficulties. Pathologic liver changes have been noted in rats fed as little as 5 ppm of mirex for 5-1/2 months.

Mirex has the highest chronicity factor observed in any pesticide examined to date. This fact, coupled with slow excretion and lack of metabolism indicate that mirex is highly cumulative in effect. Metabolic studies have shown a pattern of steady accumulation in the organs and a very slow rate of dissipation following withdrawal of mirex treatment in both mammal species tested (rats and rhesus monkeys). No significant metabolism of mirex within the body has been documented.

Other chronic effects of mirex are induction of hepatic microsomal enzymes, inhibition of several liver enzymes (LDH, MDH, SDHH, GOT, and GPT) and proliferation of smooth endoplasmic reticulum.

Mirex and Kepone produce similar adverse reproductive effects in numerous species tested. These include reductions in fertility and litter size, visceral anomalies, elevated pesticide levels in offspring (resulting from placental and lactogenic transfer) and decreases in birth weight and survival of young.

Mirex has been shown to be tumorigenic, and possibly carcinogenic in mice, producing chiefly liver tumors, however, reticular cell sarcomas and pulmonary adenomas were also seen. In oral administration tests, mirex possesses nearly the same carcinogenic potency as seven known carcinogens when tested under the same protocol (relative risk for mirex was 0.945).

Kepone, in both oral and dermal administrations is more acutely toxic than mirex in mammalian species. The most characteristic symptom of acute or subacute Kepone intoxication in mammals is severe and persistent tremor. Enlargement and congestion of the liver, weight depression, increased oxygen consumption and urinary excretion of protein and sugar were reported in chronic toxicity studies on rodents and dogs.

Like mirex, Kepone accumulates in mammalian tissues, and excretion following termination of dietary exposure is slow. No metabolites of Kepone have been detected in various organs of Kepone-fed mice; such a finding is suggestive of great biological stability of Kepone once it enters the body. Reproductive effects, placental and lactogenic transfer, and teratogenic effects are similar to those described for mirex. Kepone is a documented carcinogen in rats and mice. Following long-term exposures (80 weeks) significant increases in hepatocellular carcinomas were observed in tests conducted by the National Cancer Institute. Tests conducted by Allied Chemical Corporation also indicated that Kepone is oncogenic, however, the findings of that study were somewhat more equivocal.

5.2 AQUATIC BIOTA

5.2.1 Mirex

The ecological significance of mirex incorporation into the aquatic environment does not necessarily depend upon the amount of the residue that enters the water. What is ecologically and biologically significant is the manner in which mirex is taken up by aquatic organisms and the effects of this pesticide on biota chronically exposed to low concentrations of the chemical (de La Cruz and Naqvi, 1973).

Chlorinated hydrocarbons, including mirex and Kepone, have low solubilities in water; the solubility of mirex in fresh water does not exceed 1 ppb, and may be 0.2 ppb or less in seawater (Section 2.3.1.1). Rather than remaining suspended in water, mirex is rapidly adsorbed on various organic and inorganic particles which settle to become part of the sediments. Brown, et al., (1975) showed that at least 95 percent of the mirex in aqueous solutions is removed by adsorption on organic matter, kaolinite clay and montmorillonite clay within 2, 7, and 30 days, respectively. Therefore, in nature, mirex will not remain long in the water column but instead will be removed to the sediments where it may have an appreciable lifetime. At environmentally realistic concentrations mirex does not appear to be directly toxic to marine algae and freshwater phytoplankton in terms of population growth, oxygen evolution, or primary productivity (Brown, et al., 1975; Hollister, et al., 1975). Several types of algae have shown a propensity to accumulate mirex from water, indicating that algae may act as passive transporters of mirex to consumers in the food chain. In addition, adsorption on various organic particles in the sediments results in high toxicant input to detritus feeders (Leffler, 1975).

5.2.1.1 Algae and Phytoplankton--

Little information is available on mirex (its uptake, accumulation, or effects) and algae and/or phytoplankton. Butler (1963) found that the exposure of phytoplankton to 1 ppm mirex for 4 hours reduced productivity by 28 to 46 percent. After being exposed to 1 ppm mirex for 168 hours, pure cultures of the green alga *Chlamydomonas* sp. exhibited a 55 percent reduction in photosynthesis (de La Cruz and Naqvi, 1973). In fact, net photosynthesis was reduced from 122 mg C/m^3/hr in control cultures to 55 mg C/m^3/hr in mirex-treated cultures. The respiration rate was, likewise, reduced from 362 to 259 mg C/m^3/hr. These data concur with reported findings on the effects of various pesticides on photosynthesis.

Hollister et al., (1975) exposed marine green algae (*Chlorococcum* sp., *Dunaliella tertiolecta* and *Chlamydomonas* sp.), diatoms (*Thalassiosira pseudonana*), and red algae (*Porphirdium cruentum*) to 0.2 ppb mirex to determine the effects of mirex on population growth and oxygen evolution under various conditions of salinity and nutrient concentration. (Due to its low solubility in seawater 0.2 ppb mirex has been the highest test concentration obtainable). Mirex had no significant effect on either population growth (Figure 5.1) or oxygen evolution. The same organisms were exposed to 10, 25, or 50 ppt mirex to determine if mirex can be accumulated by these algae. Eighty-eight percent of mirex was removed from the medium by *Chlorococcum* whereas *Dunaliella* and *Thalassiosira* removed about 79 percent and *Chlamydomonas'* uptake was 55 percent. *Chlorococcum* concentrated mirex 7300 X, *Dunaliella* 4100 X, *Chlamydomonas* 3200 X, and *Thalassiosira* 5000 X the concentration in the culture medium (Figure 5.2). The ability to take up and concentrate mirex indicates that these marine algae have great potential as passive transporters of this toxicant to consumer organism in the food chain.

Sikka, et al., (1976) studied the effects of mirex on the photosynthesis of green (*Ulva lactuca* and *Enteromorpha linza*) and red (*Rhodymenia pseudopalmata*) marine algae at a concentration of 10.2 ppb. This is probably an environmentally realistic mirex concentration because it did not exceed its solubility in the growth medium (synthetic seawater). The effect of mirex on the chemical composition of the seaweeds and of the uptake and metabolism of mirex by those algae was also investigated. Mirex did not adversely affect photosynthesis and the chemical composition of *Ulva*, *Enteromorpha,* and *Rhodymenia*. The three species readily removed ^{14}C-mirex from the medium (mirex concentration in the medium was 15 ppb) and continued to remove mirex up to 144 hours after treatment when the experiment was terminated. Mirex was also accumulated by all three algal species, the bioaccumulation factor ranging from about 350 to 1100. None of the test organisms was able to metabolize mirex. Although mirex did not affect the seaweeds used in this study, the ability of the algae to accumulate mirex suggests that these organisms may act as physical agents in transporting this pesticide in estuarine ecosystems.

5.2.1.2 Aquatic Invertebrates--

5.2.1.2.1 *Uptake and accumulation*--Exposure of selected aquatic invertebrates to mirex revealed species variability in uptake which was generally a function of exposure period and concentration (Ludke, et al., 1971; de La Cruz and Naqvi, 1973). Freshwater crustaceans *Orconectes mississipiensis*, crayfish; *Palaemonetes kadiakensis,* shrimp; *Hyallela azteca*, amphipod, leeches (*Placobdella rugosa*, *Glossiphona* sp.,

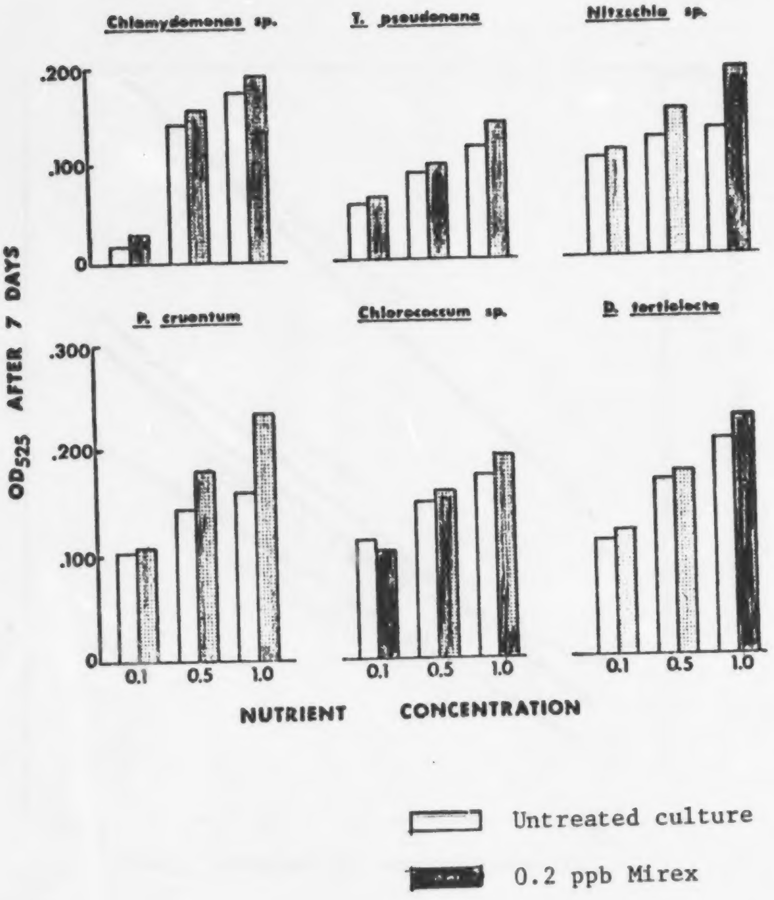

FIGURE 5.1. Comparison of cell densities at three nutrient concentrations of untreated cultures and those grown in 0.2 ppb mirex. Adapted from Hollister, et al., (1975).

Source: Hollister, et al (1975). Reprinted, with permission, from Bull. Environmental Contamination and Toxicology. (c) Springer-Verlag New York Inc. (1975).

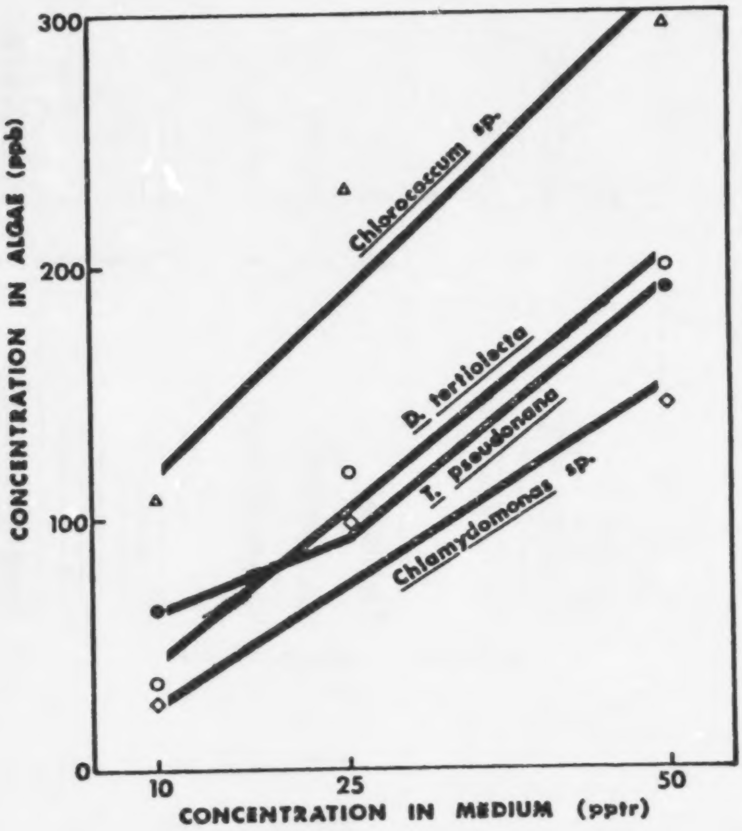

FIGURE 5.2. Uptake of mirex by algal populations after seven days exposure.

[a]Source: Hollister, et al (1975). Reprinted, with permission, from Bull. Environmental Contamination and Toxicology. (c) Springer-Verlag New York Inc. (1975).

Eropobdella punctata), and dragonfly naiads, *Macromia* sp. were exposed for 24 to 672 hours to aqueous test solutions formulated to contain 0.001 to 2.0 ppm mirex. There was great species variability in the rate of uptake of mirex. Tests conducted at the 2-ppm level for 48 hours showed an uptake of 10.0 ppm in *Palaemonetes kadiakensis* and 11.0 ppm in *Orconectes mississippiensis* which was more than twice the level of mirex residue detected in the three species of leeches (*Erpobdella punctata*, 1.9 ppm; *Placobdella rgosa*, 4.9 ppm; and *Glossiphonia* sp., 5.1 ppm). Mirex uptakes in *Orconectes*, *Placobdella*, and *Macromia* showed differences in residue levels with respect to concentration and duration of exposures (de La Cruz and Naqvi, 1973).

Collins, et al., (1975) found 0.40 ppm mirex in crayfish (*Camarellus schufeldtii*) 5 days after an aerial application of mirex bait (1.4 kg/ha, 1.25 lb/ac) to a 1.22 hectare (3-acre) fish pond and the surrounding drainage area. After 16 months, residues as high as 0.02 ppm were still present. In that same study, dragonfly nymphs (5 or more undetermined species) contained 0.70 ppm mirex 5 days following the aerial application. Sixteen months later, a concentration of 0.03 ppm mirex was found in the nymphs.

Thirty-five freshwater crayfish (*Procambarus blandingi*), about 1 cm long, were exposed to 35 granules of mirex bait (0.3 percent active ingredient). Triplicate samples (6/sample) had body residues of 1.60, 1.41, and 1.34 ppm mirex (Ludke, et al., 1971). Substantial amounts of mirex obviously leached from the granular bait into the water. The animals accumulated a residue (average of 1.45 ppm) 16,860-fold greater than that in the water (an average of 0.86 ppb of mirex had leached into the water).

Tissue residue analyses of *P. blandingi* which had been directly fed two granules of mirex bait revealed an increase in residue concentration with length of exposure (Ludke, et al., 1971). Mirex residues in crayfish bodies were from 940-fold to 27,200-fold greater than the concentration in the surrounding water. Mirex concentration in the digestive glands from four individuals living after eight days of exposure was 126,600-fold greater than that in the water. Body residue levels of mirex ranged from 3.9 to 6.1 ppm and correlated with the period of highest increase in mortality (2 and 3 days post-feeding). These data show that crayfish concentrate mirex rather efficiently from bait granules and emphasize the potential hazard of biological magnification of this toxicant.

Markin, et al., (1972), in light of the laboratory investigations of the sensitivity of crayfish to mirex and the amount of mirex used in crayfish-growing areas of Louisiana, examined levels of mirex in the edible red crayfish to determine whether continued use of mirex could threaten this locally important industry. Secondly, they wanted to determine whether mirex residues in crayfish in treated areas approach the levels found to affect crayfish under laboratory conditions. Samples were collected at the peak of the crayfish harvests in May, 1971. All collections were made from ponds, canals, and bayous which were adjacent to pastures that had a history of mirex bait application. Seven of 28 samples showed detectable residues of mirex, but these residues could neither be associated with the frequency of the treatment nor the type of crayfish habitat. The few residues which were detected ranged from 0.01 to 0.07 ppm, well below the 1- to 8-ppm level reported to kill juvenile crayfish in the laboratory.

In 1969, a cooperative USDA and USDI experiment was set up at Charleston,, South Carolina, to study effects of mirex on crab and shrimp populations (Markin, et al., 1974). Mirex was applied three times to a 2-square mile area of marshland and estuaries. Biweekly samples were collected within the treated areas during the application period and for 3 years afterwards, and analyzed for mirex residues. Mirex residues over the period ranged from 1.54 ppm 1 week after the first treatment to less than 0.001 ppm (negligible) 3 years after the third and final application.

Laboratory experiments were conducted to determine the effects of mirex and other pesticides on the larval development of blue crabs, Callinectes sapidus, from the time of hatching until the first crab stage was reached (Bookhout and Costlow, 1976). Larvae of three mother crabs were reared in mass cultures consisting of an acetone control and 0.01, 0.1, 1.0, and 10.0 ppb mirex (added as an acetone solution) to obtain enough wet weight of larvae (0.30 to 0.20 g) for residue analysis. Residue analyses were made to assess relationship between length of time in each concentration and residues of mirex and between mirex concentration and eventual residue concentration.

No detectable mirex (5 ppb) was found in freshly hatched larvae in seawater, nor in 5- and 15-day larvae, megalopa, or in first- and second-stage crabs which had been reared in the control containing only acetone. Larvae reared in a range of concentrations of mirex from 0.01 to 10.0 ppb showed increased residues of mirex with increased concentrations (Table 5.1). According to these residue analyses, the biological magnification was greatest in larvae reared in 0.01 ppb and decreased with concentration from 0.1 to 10.0 ppb mirex. These seemingly anomalous results are probably explained by the fact that crabs, being sensitive to mirex, are killed at the higher concentrations. Typically, however, only live organisms are assayed in these experiments.

Fourth and fifth stage crabs (Rhithropanopeus harrisii) contained 0.13 ppm mirex after a 48-day exposure to 0.1 ppb of the pesticide. No residues were detected in those R. harrisii crabs, reared in a solution of 0.01 ppb mirex. The presence of 0.015 ppm mirex in crabs (Menippe mercenaria) reared in 0.01 ppb mirex indicates that during these developmental stages crabs absorbed mirex during development to the second crab stage and concentrated it 1500 X. Crabs reared in 0.1 ppb mirex during development to the second crab stage absorbed and concentrated it 2400 X (0.24 ppm) (Bookout, et al., 1972).

TABLE 5.1. MIREX RESIDUES IN BLUE CRABS AND THEIR LARVAE[a]

Sample Identification and Treatment	Mirex, ppb
First-day larvae - Seawater	<5
5-day larvae - Acetone Control	<5
15-day larvae - Acetone Control	<5
Megalopa - Acetone Control	<5
Crabs, 1st and 2nd - Acetone Control	<5
5-day larvae - 0.01 ppb Mirex	11
15-day larvae - 0.01 ppb Mirex	30
Megalopa - 0.01 ppb Mirex	20
Crabs, 1st and 2nd - 0.01 ppb Mirex	9
5-day larvae - 0.10 ppb Mirex	33
15-day larvae - 0.10 ppb Mirex	70
Megalopa - 0.10 ppb Mirex	65
Crabs, 1st and 2nd - 0.10 ppb Mirex	77
5-day larvae - 1.0 ppb Mirex	301
8-day larvae - 1.0 ppb Mirex	406
5-day larvae - 10.0 ppb Mirex	1620
8-day larvae - 10.0 ppb Mirex	1370
Mother Crab Cs II - Seawater	10
Mother Crab Cs III - Seawater	<5
Mother Crab Cs V - Seawater	<5

[a] Adapted from Bookhout and Costlow (1976).

Juvenile pink shrimp, *Penaeus duorarum*, were exposed to 0.1 ppb of technical-grade mirex in flowing seawater for 3 weeks (Lowe, et al., 1971). Residue analyses of composite liver samples and remaining tissues were conducted after the crabs were placed in mirex-free seawater for 2 weeks. The livers (hepatopancreases) contained 2.4 ppm of mirex; the remaining tissues, 0.32 ppm. A composite sample of five whole shrimp contained 0.26 ppm of mirex.

Five juvenile blue crabs (*Callinectes sapidus*) were fed one fish (0.4 microgram mirex) a day for 5 days and uncontaminated fish thereafter (Lowe, et al., 1971). Three of the crabs, moribund or dead 3 weeks after receiving the fifth contaminated fish, contained 0.13, 0.22, and 0.25 ppm (0.85, 0.48, and 0.78 micrograms) of mirex. The two remaining crabs alive for 5 weeks after eating the fifth contaminated fish contained 0.10 and 0.13 ppm (0.37 and 0.57 micrograms) mirex. Another group of five juvenile blue crabs was fed only one juvenile fish containing 1.0 ppm of mirex per crab and uncontaminated fish thereafter. Sixty days later, four of the crabs were still alive and contained 0.045, 0.025, 0.052 and 0.027 ppm (0.23, 0.14, 0.19, and 0.11 micrograms) of mirex, about one-half the total amount of mirex ingested with the single contaminated fish (Lowe, et al., 1971). In another experiment, 25 juvenile crabs were placed in an aquarium through which flowing seawater passed. Each crab received one particle of mirex bait. Four weeks after the bait was fed to the crabs, residue analyses were conducted on individual crabs; the average mirex content was 0.99 ppm (Lowe, et al., 1971).

Schoor and Newman (1976) exposed adult polychaete worms (*Arenicola cristata*) to mirex bait granules equivalent to five times the field rate application (1.40 kg of 0.3 percent mirex/hectare) in a modified airlift column. Egg masses appeared in the treated tank on days 4 and 23. The exposure was terminated on day 30 and both the treated and control tanks were observed for another 45 days. Twelve swimming juvenile worms were analyzed for mirex on day 63. Their whole-body residue was 60 ppb of live weight. The experiment was terminated on day 75 and the original adult worms were analyzed. The whole-body mirex residue of the adults was 500 ppb (dry weight). Aside from predation on adult worms, swimming juvenile lugworms could transmit mirex to predators,which is an example of biological feedback. (Effects are described in Section 5.2.1.2.2).

5.2.1.2.2 *Effects*--Two species of freshwater crayfish (*Procambarus blandingi* and *P. Hayi* proved to be extremely sensitive to mirex through direct and indirect exposures under laboratory conditions (Ludke, et al., 1971). Of all the size groups tested, third instar crayfish were most sensitive to the toxicant. Delayed mortalities resulted from both low concentrations and short duration exposures to aqueous mirex as well as to oral administration in all size classes of crayfish used. Mortality among juvenile crayfish (*P. blandingi*) approached 100 percent in 5 days upon exposure to 1 ppb mirex for 144 hours (Table 5.2). *P. Hayi* exhibited 65 and 71 percent mortality, respectively, 4 days following the end of a 48-hour exposure to 0.1 and 0.5 ppb mirex.

TABLE 5.2. PERCENT MORTALITY OF P. BLANDINGI AND P. HAYI FOLLOWING INITIAL EXPOSURE TO VARIOUS CONCENTRATIONS OF MIREX[a]

Concentration, ppb	Exposure Time, hr	Number Tested	Mortality, Percent: At End of Exposure	At Days Following End of Exposure: 4	5	10
		P. Blandingi[b]				
1	144	30	0	--	95	95
5	6	30	0	--	2	26
5	24	30	6	--	13	50
5	58	40	5	--	76	98
		P. Hayi[c]				
0.1	48	30	19	65	--	--
0.5	48	30	12	71	--	--

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[a] Source: Ludke, et al. (1971). Reprinted, with permission, from Bull. Environmental Contamination and Toxicology. (c) Verlag-Springer New York, Inc. (1971).

[b] Average length 1.5 cm.

[c] Average length 0.6 m.

Feeding tests revealed that juvenile and adult crayfish (*P. blandingi*) suffered high mortalities after consuming extremely small amounts (1 or 2 granules) of mirex bait (0.3 percent active ingredient). The greatest mortality occurred during the second and third day after feeding. Some adult *P. hayi* were fed mirex bait-fed mosquitofish for 9 days and subsequently fed untreated mosquitofish for 50 days. Four of the five crayfish died. The high mortality in those individuals fed contaminated mosquitofish emphasize the potential for biological magnification (Ludke, et al., 1971).

Although Ludke, et al., (1971) found *P. blandingi* and *P. hayi* to be extremely sensitive to mirex under laboratory conditions, an earlier study of adult crayfish had indicated that they were not sensitive to mirex at the concentrations used in large-scale fire ant control projects (Muncy and Oliver, 1963). Despite evidence of extreme sensitivity of crayfish to mirex under laboratory conditions, field studies and monitoring in the estuarine environment revealed no evidence of massive die-offs or noticeable population declines. In fact, doubt has been cast on the present use of mirex being a threat to the crayfish industry (Markin, et al., 1972).

Exposure to mirex under laboratory conditions affects certain estuarine crustaceans by causing irritability, loss of equilibrium, undirected movement, paralysis, and even death. The onset of symptoms depends on the level of exposure or amount of mirex ingested, temperature, and, in some cases, the age or size of the animal being tested. The most susceptible species were found to be juvenile brown and pink shrimp and juvenile blue crabs (Lowe, et al., 1971; Bookout and Costlow, 1976; Leffler, 1975).

For blue crabs, Leffler (1975) considers mirex to be more hazardous than DDT. This is supported by a laboratory study in which the effects of ingested mirex on the metabolic rate, ionic and osmotic regulation, ability to autotomize limbs, and carapace thickness of juvenile blue crabs (*Callinectes sapidus*) were determined. Food particles, treated with acetone solutions of mirex, were placed directly against the crab's mouthparts and were devoured within a couple of minutes.

Ingested mirex was highly toxic to the juvenile crabs. Toxic responses (convulsions and death) were most pronouunced in media with very high or very low salinities. Internal mirex concentrations in crabs exhibiting toxic responses averaged 0.42 0.05 ppm after three to four weekly feedings of 0.14 microgram mirex each. Pronounced elevations of metabolic rates were induced by high subacute internal levels (0.19 0.03 ppm) of mirex. Mirex concentrations as low as 0.02 ppm in body tissues also caused significant metabolic rate elevations.

Mirex had no significant effect on the ionic composition of the hemolymph or its total osmotic concentration at subacute internal levels of 0.19

ppm mirex. The same mirex concentrations decreased the carapace thickness/width ratio of molt cycle stage C_4 crabs. Internal mirex concentrations of 0.02 ppm and above inhibited the autotomy reflex, In fact, only 29 percent of mirex-treated crabs with a damaged limb autotomized that limb. The above data verify that 0.14 microgram mirex ingested by blue crabs from a food source in the environment may result in the adverse effects outlined above.

Bookhout and Costlow (1976) exposed blue crab larvae to mirex concentrations of 0.01 to 10.0 ppb to examine developmental effects. The mirex levels had no appreciable effect on day-to-day survival of two replicate series of larval crabs for five days after hatching. After a 5-day period of delayed mortality (observed at all experimental concentrations of mirex) concentrations of 0.01 and 0.1 ppb were shown to be sublethal while 1 and 10 ppb were acutely toxic.

In one series of tests with blue crab larvae, 0.5 percent of 200 larvae reached the crab stage in 1.0 ppb mirex but no larvae from a second series survived beyond 20 days. Also, in the two series of crab larval tests, the duration from hatching to megalopa and to the first crab stage in 0.01 and 0.1 ppb mirex showed no statistical difference. In the acutely toxic concentration of 1.0 ppb mirex only 2 percent of one group of larvae reached the megalopa stage, taking 11 days longer to do so than those reared in the sublethal concentrations.

These investigators concluded that blue crab larvae are about as sensitive to mirex as juvenile blue crabs. Again, the sensitivity to mirex of the blue crab larval and juvenile life stages is extremely significant because their survival success determines the fate of entire blue crab populations.

In a study of the effects of mirex on the complete larval development of two crabs, *Rhithropanopeus harrisii* and *Menippe mercenaria*, Bookhout, et al., (1972) obtained results quite similar to those of Bookhout and Costlow (1976). The same mirex concentrations were found to be sublethal and acutely toxic. Delayed mortality was also noted in the early stages of zoeal development. Larvae of *Menippe* were much more sensitive to various concentrations of mirex than were those of *Rhithropanopeus*, especially in the megalopal stage. However, survival was reduced in relation to increased mirex concentrations in both genera. The duration of development of *Rhithropanopeus* was extended with increasing mirex concentrations. In the case of *Menippe*, the duration of stages fluctuated but showed very little evidence of becoming longer as mirex concentrations increased.

Lowe, et al., (1971) cited an earlier study conducted in their laboratory in which juvenile blue crabs and juvenile pink shrimp (*Penaeus duorarum* showed no symptoms of poisoning during a 96-hour exposure to a suspension of 0.1 ppm technical mirex in flowing seawater. These organisms, however, did become irritated and paralyzed, and died within

18 days after being placed in mirex-free water. This serves as an additional illustration of delayed mortality among mirex-exposed crustaceans.

In the above study, Lowe, et al., found that exposure to one particle of bait (ca. 4.4 micrograms mirex) per juvenile blue crab caused significant mortality (53 to 84 percent) by the end of the 4-week experimental period. Fiddler crabs (Uca pugilator) were also susceptible to poisoning by mirex. Exposures to 50 mg of mirex bait (which is equivalent to a field application of 1.4 kg/ha) caused 73 percent mortality or paralysis within two weeks.

Juvenile brown shrimp (Penaeus aztecus) and grass shrimp (Palaemonetes pugio) died after exposure to mirex bait (0.3 percent active ingredient) at levels as low as one particle of bait per shrimp in standing seawater. Within 96 hours, the above exposure resulted in 60 and 70 percent mortality, respectively, in brown and grass shrimp (Lowe, et al., 1971).

Juvenile pink shrimp (Penaeus duorarum) are extremely sensitive to mirex dissolved in seawater. Twenty-five percent (9 of 36) of the shrimp became irritated and died during a 7-day exposure to 1.0 ppb of technical mirex in flowing seawater. The remaining 75 percent died within 4 days after the exposure ended (Lowe, et al., 1971). A second group of shrimp was exposed to 0.1 ppb of technical mirex in flowing seawater for 3 weeks. Only 11 percent (4 of 36) of the shrimp died during the 3-week exposure, but several more showed symptoms of mirex poisoning with 9 dying during the 2-week posttreatment period in mirex-free water. The juvenile pink shrimp clearly exhibited the phenomenon of delayed mortality.

Tagatz (1976) designed an experimental estuarine ecosystem consisting of turtle grass (Thalassia testudinum), grass shrimp (Palaemonetes vulgaris) and pinfish (Lagodon rhomboides) to determine the distribution and biological effects of mirex. Sublethal concentrations were used in tests to assess the effects of mirex in terms of an alteration of predator-prey interactions. The experiments provided data on grass shrimp survival after one, two, and three days of predation by pinfish. More deaths due to predation occurred in the mirex-treated tanks. In all experiments, an alteration of predator-prey could be interpreted as an alteration of normal behavior of either shrimp or pinfish by mirex. The author believed that the behavior of only the grass shrimp was altered.

Adult lugworms (Arenicola cristata) were exposed to mirex bait granules (0.3 percent active ingredient) for 30 days and observations were made for an additional 45 days to determine the effects of mirex on the activity of this benthic organism (Schoor and Newman, 1976). Egg masses were deposited during days 4 and 23 of the exposure. Free-swimming juveniles and juveniles burrowing in the upper layers of the substrate of the aquarium were observed. Observed feeding behavior and general activity were considerably less among the mirex-exposed organisms compared with those of the controls. Overall, low concentrations (0.062 to 0.003 ppb) of mirex in water decreased behavioral activity as measured by surface activity.

On day 75 (end of the experiment), reduced activity was observed among the experimentals and controls; that of the exposed worms was much lower. Considerable destruction of the layered substratum occurred, especially in the upper half. Mixing due to the burrowing activity of the worms was indicated. Sand samples taken from the top of the substratum contained 0.54 ppb mirex (dry sand); from the middle, 0.34 ppb; and from the bottom, 0.08 ppb. In contrast, in the absence of worms, mirex residue in the same substratum was found only in the top 2 cm (about 1.5 ppb) after a 17-day exposure·to similar concentrations of mirex in water (average of 0.025 ppb). This definitely indicates that the feeding and burrowing activity of Arenicola can affect distribution of mirex in the substrate. (Mirex accumulation in the worms is described in Section 5.2.1.2.1).

Oxygen consumption of the pond snail, Physa gyrina, exposed for 3 days to very low mirex concentrations (0.008 to 0.07 ppm) leached from mirex bait increased to a maximum of 62 percent. Oxygen consumption dropped by 44 percent at the 1 ppm concentration. This initial increase in oxygen consumption is considered a normal physiological response to toxic stress (de La Cruz and Naqvi, 1973) It should be noted that the mirex concentrations employed in this experiment exceeded normal saturation (0.2-1.0 ppb) by as much as 70 times, and expected environmental concentrations by at least a factor of 2000.

5.2.1.3 Fish—

5.2.1.3.1 Uptake and accumulation--Mirex residues can usually be detected in most fish taken from waters near treated areas, including edible species such as bass, bream, catfish, and mullet. Bass and bream usually contain less than 1.0 ppm in their edible portions. Wild catfish and mullet have been found to contain residues as high as 1 to 5 ppm (Mirex Advisory Committee, 1972).

Hawthorne, et al., (1974) sampled and analyzed commercially raised catfish from Mississippi and southern Arkansas areas in which mirex has been used extensively for fire ant control. Fifty samples, including 25 from nonexposed areas, were analyzed. No mirex was found at the detection level of 0.01 ppm; however, samples from both areas did contain extensive residues of other chlorinated pesticides (e.g., dieldrin, endrin, and DDT and its analogs). The lack of detectable residues is an indication that there was not widespread contamination of catfish. These investigators suggest that mirex is probably one of the least likely of the chlorinated pesticides to reach humans through consumption of catfish.

In another study, a 1.22-hectare (3-acre) fish pond and surrounding drainage area were treated with mirex (1.7 g/ac) six days after fingerling and adult channel catfish (*Ictalurus punctatus*) had been released into the pond (Collins, et al., 1973). Wire cages were used to confine 50 of the larger fish which were periodically fed commercial catfish food. Uncaged fish fed on organisms (minnows, crayfish, insect larvae) which inhabited the pond. A buildup of mirex residues occurred in uncaged fish whereas the larger caged fish acquired no detectable mirex and only minute levels of mirex were detected in caged fingerlings. Mirex residues in the uncaged fish were not detectable 5 days after teatment but began to increase reaching a maximum of 0.65 ppm 6 months after treatment. Sixteen months after treatment uncaged fish contained 0.44 ppm mirex.

Collins, et al., (1973) believed that if a true commercial catfish pond had been used in their experiment, residues would not have been as high. Routine monitoring by these researchers showed mirex residues ranging from 0.008 to 2.59 ppm in wild catfish from areas which received a blanket application of mirex bait, whereas commercially grown fish from ponds contained no detectable mirex residues.

Before the study by Collins, et al., (1973), minnows (*Opsopoeodus emiliae*) and mosquitofish (*Gambusia affinis*) were the only fish in the 1.22 hectare (3-acre) study pond. These fish contained detectable mirex residues which decreased substantially one year after the mirex application, but were still detectable 16 months after treatment. The mosquitofish contained 0.08 ppm mirex 38 days after treatment, and 0.07 ppm at 16 months posttreatment. Minnows contained 0.11 ppm 38 days after treatment; 16 months after treatment minnows contained 0.07 ppm.

Mirex bait (0.3 percent) was applied three times at a rate of 1.4 kg/ha to four ponds containing channel catfish (Hyde, et al., 1974). The first application was made 8 days after fingerling catfish were placed in the ponds. Catfish and specimens from a natural population of *Lepomis* spp. were collected at each subsequent application and about 2-1/2 months after the final application. Relatively small quantities of mirex were detected in the catfish subsequent to the three applications. Mirex was detected in the catfish muscle from treated ponds at 0.01, 0.03, and 0.015 ppm for the first to the third sampling date, respectively. In catfish fat, the mirex levels were 0.10 ppm 109 days after the second mirex application and 0.255 ppm 76 days after the third application. Levels of mirex in *Lepomis* spp. were 0.01 ppm 107 days after the first mirex application and 0.06 ppm 109 days after the second application. A composite sample of *Lepomis* contained 0.02 ppm mirex 76 days after the third mirex application.

Hyde, et al., (1974) attributed the relatively low residues to a commercial diet being fed to the catfish and *Lepomis*. The higher mirex level in *Lepomis* may reflect a tendency of these species to rely on a natural food supply and, thereby, accumulate more mirex from food complexes. The relatively high concentration of mirex in catfish fat is related to the affinity of mirex for lipid material.

Pimentel (1971) has cited a study in which bluegills (Lepomis macrochirus) concentrated mirex to a level of 6.82 ppm when living in pond water containing 1 ppm mirex.

Lee, et al., (1975) exposed adult striped mullet (Mugil cephalus) to 0.5 ppm mirex for 96 hours in a continuous-flow bioassay system. Mirex became most concentrated in the visceral organs. The levels of mirex from the 96-hour samples were: 21.5 ppm (wet weight) from viscera; 2.0 ppm from hearts and gills; and 1.4 ppm from skin and muscle. Exposures of juveniles and adults to 0.01 to 10.0 ppm mirex in a continuous-flow bioassay system resulted in higher residue levels in the adults (Table 5.3). This is presumably due to the higher proportion of body fat in adults than in juveniles. The amount of mirex in the fish increased with increasing mirex concentration in test water.

Twenty-five juvenile pinfish lived for 5 months on a diet that contained approximately 20 ppm technical mirex. They exhibited no symptoms of poisoning but concentrated high residues (30 to 40 ppm) of mirex in body tissues. The fish contained an average of 18 ppm mirex 8 weeks after the exposure ended which indicates that mirex is not easily metabolized by juvenile pinfish (Lowe, et al., 1971).

5.2.1.3.2 Effects--The limited data on the effects of mirex on fish concern growth, oxygen consumption, and pathologic developments.

The effects of mirex on bluegills (Lepomis macrochirus) and goldfish (Carassius auratus) were studied in three experiments in which the fish were exposed either by feeding them a mirex-treated diet, or by treating the holding ponds with a mirex formulation (Van Valin, et al., 1968). There were no mirex-related mortalities among bluegills from single mirex applications (0.0013 and 1.0 ppm) to holding ponds. However, the number of fish remaining at the end of this first experiment in each pond was well below the theoretically possible 380 fish. Pathologic examinations did not reveal any histologic lesions associated with mirex applications.

Goldfish mortality was noted throughout the second experiment which also involved mirex treatment (0.1 and 1.0 ppm) of holding ponds. The remaining goldfish were suffering from a mycobacterial infection. Those that died were emaciated, lacked the slime layers; had roughened skin with many protruding scales, and exhibited edematous gill changes. In addition, a granulomatous kidney lesion, first noted in a single specimen 28 days posttreatment in the 1.0 ppm mirex-treated pond, gradually increased until it was seen in 75 to 100 percent of all specimens by day 224. The severity of infection in individual goldfish was closely related to the treatment level. Gall bladders were also distended to two or three times the circumference of those from fish from the control ponds (Van Valin, et al., 1968).

TABLE 5.3. CONCENTRATION OF MIREX ACCUMULATED BY STRIPED MULLET EXPOSED TO MIREX IN WATER FOR 96 HOURS IN A CONTINUOUS-FLOW BIOASSAY SYSTEM[a,b]

Age Group	Mirex Concentration in Water, ppm		Mirex Residue, ppm[c,d]	
	Added	Measured	Mean	S.D.
Juvenile	0.01	0.010	0.17	0.02
	0.1	0.104	0.85	0.31
	1.0	1.108	3.90	0.16
	10.0	5.180	17.81	2.47
Adult	0.01	0.012	0.38	0.07
	0.1	0.107	1.02	0.17
	1.0	1.033	6.15	1.91
	10.0	9.540	37.30	4.83

[a] Source: Lee, et al. (1975). Reprinted, with permission, from Bull. of Environmental Contamination and Toxicology. (c) Springer-Verlag New York, Inc. (1975).

[b] Number of fish used for analysis: 10 juveniles, and 2 adults per replicate aquarium.

[c] Concentration in mullet expressed as whole-body wet weight.

[d] Duplicate samples per replicate were taken for analysis. Mean values were obtained with the data from eight separate analyses for each concentration.

There was no relationship found between growth and degree of exposure in the experiments involving holding pond treatments. Growth was very slow among bluegills and the average weight of goldfish declined. Exposure to mirex had no apparent effect on the spawning success of fish in either experiment.

Mortality in the feeding experiment was not related to the level of exposure. No morphologic or systemic changes appeared to be associated with the exposure to mirex. For bluegills fed the highest level of mirex (5 ppm) growth was affected. Their average weight was 18.3 grams 168 days after treatment, as compared with 27.0 and 23.5 grams, respectively, at the 1- and 3-ppm treatment levels for the same time period. Parasitism was a problem with many of the fish exposed to mirex through feeding. However, there was no apparent relationship between the level of mirex intake and degree of parasitism (Van Valin, 1968).

The longer channel catfish were exposed to mirex, the greater the observed mean weight disparity (Hyde, et al., 1974). Catfish from ponds that had received three applications of 0.3 percent mirex bait at the rate of 1.4 kg/ha weighed an average of 10.8, 16.8, and 40.8 g less than fish from untreated ponds.

Mosquitofish (*Gambusia affinis*) and bluegills (*Lepomis macrochirus*) were exposed to mirex leached from fire ant baits. Both *Gambusia* and *Lepomis* exhibited insignificant increases in oxygen consumption after 3 weeks exposure in water containing 1 ppm mirex. Oxygen consumption by mirex-treated fish decreased slightly from the fourth week of exposure and stabilized beginning the fifth week for *Lepomis* and on the seventh week for *Gambusia*. As stated earlier an initial increase in oxygen consumption is considered to be a normal physiological response to toxic stress (de La Cruz and Naqvi, 1973).

Young striped mullet juveniles (*Mugil cephalus*), 20 to 43 mm, were more susceptible to mirex exposure than older juveniles, 70 to 150 mm, or adults, 260 to 380 mm (Lee, et al., 1975). Exposures to mirex levels of 0.01, 0.1, 1.0, and 10.0 ppm during 96-hour continuous-flow bioassays resulted in no mortalities among older juveniles and adults. For young juveniles, mortality was higher in the two intermediate concentrations (0.10 and 1.0 ppm mirex) 26.9 percent and 32.1 percent. Concentrations at either extreme produced less mortality; 0.01 ppm resulted in 6.4 percent mortality and 10.0 ppm resulted in only 9 percent mortality. The authors were unsure whether these results were a result of a specific action of mirex on striped mullet or due to experimental variation.

5.2.1.4 Food Chains--

From the data currently available, it is evident that mirex is reaching some nontarget organisms as a result of previous large-scale applications for fire ant control. It is also apparent that mirex can be transferred through simple two-level food chains established in the laboratory (Lowe, et al., 1971). Grass shrimp (*Palaemonetes pugio* and *P. Vulgaris*) were individually fed one particle of mirex bait in standing seawater. Those which died (more than 50 percent in 96 hours) were later fed to juvenile blue crabs being held in compartmented, flowing seawater aquaria. All the crabs died within 14 days after feeding on the contaminated shrimp. The number of shrimp eaten by each crab ranged from one to four. A pooled sample of ten grass shrimp carcasses contained 1.1 ppm (2.5 g) of mirex while a pooled sample of seven crabs contained 0.16 ppm (1.9 g) of mirex. Lowe, et al., also found that juvenile blue crabs accumulated mirex residues after having fed on fish containing the insecticide (Section 5.2.1.2.1).

The food chain also appears to be the mode of transport of mirex in natural populations. Collins, et al., (1973) treated a 1.22-hectare (3 acre) fish pond and the surrounding drainage area with mirex (1.7 g mirex/ac). They found a buildup of mirex residues in uncaged channel catfish (Section 5.2.1.3.1) whereas only minute levels of mirex were detected in the caged fingerling catfish. The explanation for such an occurrence was that caged fish which were fed a diet of commercial catfish food, did not have a ready access to the natural food chain (minnows, crayfish, insect larvae). Had the uncaged fish obtained their residues by directly feeding on mirex bait granules, residues would have been quite high soon after treatment and would have decreased with time. Instead, mirex residues were not detectable 5 days after treatment, but then gradually increased with time, reaching a maximum concentration six months after treatment, and were still present 16 months after treatment. The data from this study and that of Hawthorne, et al., (1974) show that mirex is unlikely to reach humans through the consumption of commercially raised catfish.

Collins, et al., (1974) monitored accumulation of mirex residues following application of mirex bait and corroborated the findings of earlier studies which suggested that the source of mirex residues in fish is through the food chain rather than through direct ingestion of the bait pellets. As expected, predatory species of fish such as largemouth bass contained higher mirex residues than did omnivorous species such as brown bullheads and sunfish. Gopher tortoises and other strictly herbivorous species contained significantly less mirex than did either omnivorous or carnivorous species. Regardless of trophic levels, the authors found that residues in most animals peaked 1 to 3 months following bait application and decreased afterward.

Hyde, et al., (1973a) presented disturbing evidence about the accumulation of mirex in aquatic and terrestrial food chains. An area under investigation in Louisiana received six applications of mirex bait at 1.4 kg/ha (1.25 lb/ac) over a 4-year period. Aquatic plants in this area contained negligible amounts; snails, crayfish, and fish, 0.01 to 0.75 ppm; soft-shell turtle fat, 24.8 ppm; and birds, 1.2 to 1.9 ppm; while the fat of vertebrates (raccoons) at the end of the food chain contained up to 73.9 ppm. The environmental significance of mirex residues in nontarget species is for the most part unknown. Mirex was detected in animal tissues at concentrations as high as 0.70 ppm in several species that are commonly used for human consumption. Those species include bluegills, chain pickerel, and quail.

Metcalf, et al., (1973) reported a study in which a model ecosystem was set up in an aquarium using a terrestrial-aquatic interface. Radiolabeled mirex bait was applied to sorghum seedlings in the terrestrial portion at the rate of 1.0 lb per acre (1.1 kg per hectare). Caterpillars fed on the seedling leaves until consumed; feces and the caterpillars were allowed to contaminate the aquatic portion which was stocked with algae, snails (Physa), Daphnia, mosquito larvae, and fish (Gambusia). The ecosystem was maintained for 33 days in an environmental chamber. The mirex was very stable, having a biodegradation index of 0.0145 and 0.006 in fish and snail at the end of the 33 day study. Ecological magnification (EM) values were 219 for fish and 1165 for snail (water equals 1). Bioconcentration results from the organochlorine's high lipid solubility and low water solubility. The high water insolubility is likely the driving force in lipid storage.

Mirex may be bioaccumulated in aquatic organisms directly from the water. EM factors for Daphnia, mosquito larvae, and fish in water with similar concentrations as the model ecosystem were 2200, 210, and 530, respectively. Mirex was not metabolized by the caterpillars. Mirex residue levels, as well as rates and direction of transfer in the natural food chain, are difficult to interpret or predict for several reasons:

(1) Numerous organisms (e.g., omnivores, detritus feeders, filter feeders) cannot be classified into traditional trophic levels. Feeding habits may change from larval stage(s) to adult and many organisms may be opportunistic feeders as adults.

(2) Certain species may move considerable distances (e.g., marsh birds, raccoons, blue crabs) and carry residues with them.

(3) Tidal flushing, storms, and hurricanes can suspend sediments in freshwater lotic and lentic systems and in estuaries. Spring turnover in lakes may also cause resuspension of mirex-bearing sediments.

(4) Mirex residues measured in the past may be partially confused with the polychlorinated biphenyl, Arochlor 1260, and/or Dechlorane, which is chemically identical to mirex (Mirex Advisory Committee, 1972).

5.2.2 Kepone

5.2.2.1 Algae and Phytoplankton--

Few data are available on the uptake and/or effects of Kepone on algae and phytoplankton. Walsh, et al., (1976) exposed marine green algae (*Chlorococcum* sp. and *Dunaliella tertiolecta*) and diatoms (*Nitzschia* sp. and *Thalassiosira pseudonana*) to Kepone to determine its effects on growth and its uptake by the phytoplankters. In the uptake studies, twelve cultures of each algae were grown in full-nutrient media for 6 days. These algae were then exposed to 100 ppb Kepone for 24 hours.

To determine the effects on growth, algae were grown in an artificial seawater medium of 30 parts per thousand salinity with full- and half-nutrient strengths. Technical grade Kepone in 0.1 ml acetone was added to the media each day for 7 days. Each Kepone exposure was performed three times. After a 24-hour exposure to 100 ppb Kepone, residues associated with the algae in ppm (wet weight), were: *Chlorococcum* sp., 80; *D. Tertiolecta*, 23; *Nitzschia* sp., 41; *T. pseudonana*, 52. The calculated EC_{50} values (Kepone concentrations which reduced growth by 50 percent after seven days' exposure) in ppm were: *Chlorococcum* sp.; 0.35; D. *tertiolecta*, 0.58; *Nitzschia* sp., 0.60; *Thalassiosira pseudonana*, 0.60.

Reductions of phytoplankton populations by short (4 hours) or longer (24 hours) exposures to Kepone concentrations ranging from 0.35 to 1.0 ppm could disturb or destroy the lowest level of the aquatic food chain which would, in turn, have a direct effect on productivity at other levels of the food chain. Kepone may be passed along the food chain to man as a result of being accumulated in algae. Consequently, there is the potential for Kepone being bioconcentrated in harmful proportions in man's food source (Section 5.2.2.4).

5.2.2.2 Aquatic Invertebrates--

5.2.2.2.1 *Uptake and accumulation*--Invertebrates bioconcentrate Kepone to dangerously high levels. Bioaccumulation data for various aquatic invertebrates are presented in Table 5.4. Schimmel and Wilson (1976) found that grass shrimp (*Palaemonetes pugio*) and blue crabs (*Callinectes sapidus*) concentrated Kepone when exposed to various levels during 96-hour flow-through bioassays. It should be noted that none of the exposures was representative of realistic environmental levels. The lowest Kepone exposure of 12 ppb resulted in whole-body Kepone residues of 5 ppm wet weight in grass shrimp. A whole-body Kepone residue of 0.85 ppm wet weight was detected in blue crabs exposed to 110 ppb (the lowest concentration in the experiment). Some of the published data on blue crabs appear to be misleading. For example, blue crabs have been obtained from the James River with as much as 6 ppm Kepone in their tissues. Yet, in the laboratory such levels have only been achieved when exposed to unrealistically high concentrations. Presumably crabs in their natural

TABLE 5.4. ACCUMULATION OF KEPONE BY SELECTED AQUATIC INVERTEBRATE SPECIES[a]

Species	Tissue	Accumulated Level, ppm (wet weight)	Bioconcentration Factor		Exposure Conditions[b]	
			Average	Range	Concentration, ppb	Time
Grass shrimp (*Palaemonetes pugio*)	Whole body	5.1-94.0	698	(425-933)	12-121	96 hours
		0.13	5,127		0.023	28 days
		5.2	11,425		0.4	28 days
Opossum shrimp (*Mysidopsis bahia*)	Whole body	0.2	5,962		0.026	21 days
		5.5	13,473		0.41	21 days
Blue crab (*Callinectes sapidus*)	Whole body	0.85-1.7	8.1	(6.2-10.4)	110-210	96 hours
Oyster (*Crassostrea virginica*)	Whole body	0.3	9,354		0.03	19 days
		3.6	9,278		0.39	21 days

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[a] Adapted from Bahner, et al., (1976).

[b] Seawater.

habitats obtain much of the pesticide from food or from sediments where they burrow.

Bahner, et al., (1976) reported high bioconcentration factors of 13,470; 11,430 and 9,280, respectively, for the opossum shrimp (mysid), grass shrimp, and oyster (see Section 5.2.2.4; Tables 5.4 and 5.5). The difference between the shrimp species and the oyster in the bioconcentration of Kepone has been attributed to depuration rates. Depuration of Kepone from oysters has been found to be rapid while for shrimp this process is relatively slow. Concentration factors for adult mysids (Mysidopsis bahia) exceeded 14,000 X within 11 days of exposure to 0.43 ppb, but averaged 6400 X during a 21-day exposure to 0.26 ppb Kepone (Nimmo, et al., 1976).

5.2.2.2.2 Effects--Kepone is acutely and chronically toxic to estuarine species under controlled exposure conditions in the laboratory. Nimmo, et al., (1976) conducted life-cycle bioassays on the mysid, Mysidopsis bahia, to determine the acute and chronic toxicities of Kepone. The 96-hour LC_{50} for Kepone in the mysid was 10.1 ppb at 10 to 16 parts per thousand salinity whereas the 19-day life-cycle LC_{50} was 1.4 ppb at 10 to 20 parts per thousand salinity.

The life-cycle test was long enough for the production of several broods. The average number of young per female at 20 days was 8.9 upon exposure to 0.39 ppb Kepone compared with 15.3 among the controls. Those 24- and 48-hour juveniles which were exposed to 0.39 ppb Kepone did not grow as rapidly as did the controls within the first 15 days. Even those female mysids exposed to as little as 0.072 ppb Kepone were shorter than controls. Also body size is correlated with reproductive success. It has been established with thirteen crustacean species (five of which are mysids) that the number of eggs produced is directly related to body length (Nimmo, et al., 1976).

In nature the loss of mysids due either to the direct toxic effects of Kepone and other pollutants or to the indirect effect on their growth or population size could affect the food supply of many fishes. Mysids are apparently very important components in the food chain of fishes and this might have a direct influence on fish productivity.

Schimmel and Wilson (1976) conducted flow-through bioassays to determine the acute effects of Kepone on two representative crustacean species found in the James River estuary. The representative 96-hour LC_{50} values were: grass shrimp, 121 ppb and blue crab, 210 ppb. The symptoms of Kepone poisoning exhibited by those species were lethargic behavior followed by a loss of equilibrium. The authors cited a study in which the 48-hour flow-through bioassay EC_{50} value (based on mortality or loss of equilibrium for shrimp and crabs) for Kepone toxicity was found to be 85 ppb for brown shrimp (Penaeus aztecus) exposed at a seawater temperature of 14 C. Twenty percent of the blue crabs exposed to 1000 ppb Kepone died in 48 hours.

Kepone was found to be among the chlorinated hydrocarbons that are least toxic to adult brown shrimp (*Penaeus aztecus*). A 96-hour EC_{50} value based on a loss of equilibrium was 0.70 ppm at 30 C. Exposure of juvenile blue crabs (*Callinectes sapidus*) to 1.0 ppm Kepone for 24 hours at 29 C produced only irritation (U.S. Environmental Protection Agency, 1976c).

Fiddler crabs (*Uca pugilator*) were exposed to 320, 560, 750, 1000, and 1600 ppb Kepone during 24-, 48- and 96-hour static bioassays (Heitmuller, 1975). The 24-hour LC_{50} value was greater than 1600 ppb and that for 48 hours was 1345 ppb. Mortality ranged from zero percent in nominal test concentrations (of 320 to 750 ppb) to 70 percent in 1600 ppb.

Kepone was found to be comparatively toxic to oysters, causing a 50 percent decrease in shell growth during a 96-hour exposure. The EC_{50} values were 0.057 ppm at 14 C and 19 parts per thousand salinity, and 0.015 ppm at 31 C and 25 parts per thousand salinity (U.S. Environmental Protection Agency, 1976c). A study by Butler (1963) showed 48-hour EC_{50} values (based on the inhibition of shell deposition) to be 57 and 15 ppb for eastern oysters (*Crassostrea virginica*) at seawater temperatures of 14 and 31 C, respectively.

5.2.2.3 Fish--

5.2.2.3.1 *Uptake and accumulation*--Laboratory exposures of fish species to Kepone at the EPA Laboratory at Gulf Breeze, Florida, showed Kepone to be bioaccumulative and persistent. Tissue residue analyses performed in toxicity bioassay studies by Schimmel and Wilson (1976) indicate that sheepshead minnows and spot (a commercially important food fish) bioconcentrate Kepone to excessively high levels. The authors determined that these fishes depurate Kepone at a rather slow rate as compared with that for the oyster. This accounts, at least in part, for the high Kepone levels. After three or more weeks in Kepone-free water, only 30 to 50 percent of the accumulated Kepone was lost. Bioconcentration factors were similar in both fish species. A bioconcentration factor of 1577 was reported for the sheepshead minnow upon exposure to a Kepone concentration of 7.1 ppb. The associated whole-body Kepone residue was 11.2 ppm wet weight. For spot, a bioconcentration factor of 1133 was equivalent to a whole-body residue of 1.7 ppm upon exposure to a Kepone concentration of 1.5 ppb.

Bahner, et al., (1976) found that spot bioconcentrated Kepone from seawater containing 0.029 ppb to a level of approximately 0.21 ppm. Sheepshead minnows contained approximately 0.54 ppm Kepone after 28 days exposure to seawater containing 0.05 ppb. The residues were higher in female sheepshead minnows (0.35 ppm) than in males (0.25 ppm). Kepone accumulated in edible fillets to near the whole-body concentrations in sheepshead minnows and spot. Therefore, one of the largest reserves (22 percent) of Kepone in absolute weight is in the edible portion of the contaminated fish.

Hansen, et al., (1976) found that concentration factors for adult sheepshead minnows averaged 2500 following exposure to Kepone concentrations ranging from 0.16 to 24 ppb. Again, the bioconcentration of Kepone was greater in female sheepshead. Kepone residues in dead fish were (exposure level in parentheses) 17 ppm (7.8 ppb); 10 ppm (1.9 ppb); and 3.4 ppm (0.8 ppb). Kepone residues in sheepshead eggs were similar to those in the female fish. Concentration factors in juvenile fish averaged 7200. Juvenile progeny of adult sheepshead exposed to 1.9 ppb Kepone, though hatched and grown in Kepone-free water, contained 0.13 ppm. Kepone concentations in juveniles increased with increased concentrations of Kepone in water.

5.2.2.3.2 *Effects*--Kepone was found in one study to be among the least toxic chlorinated hydrocarbons to certain estuarine fish. For juvenile white mullet (*Mugil curema*), the 24-hour LC_{50} was 0.5 ppm at 31 C; the 48-hour LC_{50} was 0.055 ppm. The longnose killifish (*Fundulus similis*) was found to have a 24-hour LC_{50} of 0.3 ppm at 31 C and a 48-hour LC_{50} of 0.084 ppm (U.S. Environmental Protection Agency, 1976c).

Hansen, et al., (1976) investigated the toxicity of Kepone to embryo, fry, juvenile and adult sheepshead minnows (*Cyprinodon variegatus*) using intermittent-flow bioassays. Thirty-two adult males and thirty-two adult females were exposed to 0.16, 0.80, 1.9, 7.8 and 24 ppb of Kepone for 28 days. Also, 10 fish were exposed to 0.05 ppb for 4 weeks. All fish exposed to 7.8 and 24 ppb died by day 15. Seventy-eight percent survived at 0.8 ppb and 20 percent at 1.9 ppb, and these exhibited symptoms of Kepone poisoning. Symptoms of poisoning were related to concentration and duration of exposure. They progressed from scoliosis, darkening of the posterior one-third of the body, hemmorhaging near the brain and at the anterior point of darkening, edema, fin rot, uncoordinated swimming, and cessation of feeding. Symptoms increased in severity and frequency before death. This occurred from 5 to 8 days after initial exposure.

Surviving adults were spawned, and embryonic development, hatching, and survival and growth of fry and juveniles were monitored in a 36-day exposure. The effects of exposure of progeny to 0.08 to 33 ppb Kepone were less severe than those observed with adult fish exposed to similar concentrations. The 36-day LC_{50} to juveniles was 4.9 ppb and the 28-day LC_{50} to adults was 1.1 ppb. The average standard length of juvenile fish was significantly reduced by exposure to 0.08 ppb Kepone; some fish developed scoliosis. Embryo survival was significantly reduced; 91 percent in controls and at 0.08 and 0.18 ppb Kepone as compared with 83 percent at 0.18 ppb Kepone. A significant number of embryos from adult fish exposed to 1.9 ppb Kepone developed abnormally and died even when incubated in Kepone-free water. Fry from embryos exposed to 6.6 or 33 ppb Kepone were visibly affected within 24 hours of hatching. Symptoms of poisoning in fry less than 1 week old included diminished activity, loss of equilibriium, cessation of feeding and emaciation. Fry more than 1 week old had symptoms identical to those in adult fish except for a lack of hemorrhaging and the presence of edema.

Sixty percent of juvenile fish which survived 36 days of exposure to 0.08 ppb Kepone had scoliosis and blackened tails. In Kepone-free water scoliosis persisted for more than ten days. These fish, spawned from adult sheepshead previously exposed to 0.05 ppb, were exposed to Kepone longer than those from embryo, fry, and juvenile exposures. These results illustrate very well how the long-term effects of Kepone on juvenile fish have been underestimated.

The predicted 96-hour LC_{50} values for exposures to Kepone of bluegills (*Lepomis macrochirus*) and rainbow trout (*Salmo gairdneri*) are 0.051 ppm and 0.036 ppm, respectively. The highest predicted concentration at which there was no discernible effect during a 96-hour bioassay was 0.024 ppm for bluegills and 0.014 ppm for rainbow trout (Bentley, 1974). The same author observed an 80 percent mortality in 96 hours at 0.065 ppm and a 48-hour LC_{50} of 0.180 ppm for bluegills. In 96 hours, there was an 80 percent mortality among rainbow trout at 0.037 ppm Kepone and total kill at 0.100 ppm during the 48-hour bioassay.

The U.S. Environmental Protection Agency (1976c) reviewed several toxicity studies involving Kepone and fish. The length of time redear sunfish (*Lepomis microlophus*) were exposed to Kepone had a much more pronounced effect than temperature. Rainbow trout are about ten times more susceptible to Kepone than are redear sunfish. The 24-hour LC_{50} for rainbow trout is 0.066 ppm, while the 24-hour LC_{50} for the redear sunfish is 0.62 ppm.

Kepone, applied at rates of 0.11 and 0.56 kg/ha (0.1 and 0.5 lb/ac) on shallow 0.025-hectare (1/16-acre) ponds, caused only 2 and 18 percent mortality, respectively, in populations of the mosquitofish (*Gambusia affinis*) after 24-hour exposures (U.S. Environmental Protection Agency, 1976c).

In vitro experiments (Desaiah and Koch, 1975) which tested the effects of Kepone on brain ATPase activity in channel catfish (*Ictalurus punctatus*) demonstrated significant inhibition of oligomycin-sensitive (mitochondrial) Mg^{2+}, oligomycin-insensitive Mg^{2+} and Na^{+}-K^{+} ATPases with increasing concentrations of Kepone and its reduction product decachloropentacyclo-decan -5-ol (DCPD). Oligomycin-insensitive Mg^{2+} ATPase activity was somewhat less sensitive to inhibition by Kepone. Comparable concentrations of Kepone (1.25 and 2.5 micro molar) produced 25.7 and 36.7 percent inhibition. The authors concluded that inhibition of the type noted above could probably result in physiological impairment due to a reduction in energy supply.

5.2.2.4 Food Chains--

Accumulation, transfer, and loss of Kepone in estuarine food chains were studied in laboratory bioassays. Kepone was bioconcentrated by oysters (*Crassostrea virginica*), mysids (*Mysidopsis bahia*), grass shrimp (*Palaemonetes pugio*), sheepshead minnows (*Cyprinodon variegatus*), and spot (*Leiostomus xanthurus*), from concentrations as low as 0.023 ppb in seawater (Bahner, et al., 1976). Bioconcentration factors for Kepone in the species are summarized in Table 5.5. All species had equilibrated tissue concentrations of Kepone within 8 to 17 days after beginning exposure to the pesticide.

Depuration of Kepone from oysters was rapid, with nondetectable concentrations (0.02 ppm) being found within seven to twenty days after exposure to Kepone ceased. Clearance of Kepone from grass shrimp and fish was relatively slow, with tissue Kepone residues decreasing 30 to 50 percent in 24 to 28 days.

Oysters, fed the green alga *Chlorococcum* which contained an average of 34 ppm Kepone, bioaccumulated 0.21 ppm Kepone (wet tissue) in 14 days and in Kepone-free water depurated Kepone to below detectable concentrations within 10 days. Residues in composite samples of feces and pseudofeces from these oysters averaged 1.78 ppm Kepone (dry weight).

Spot accumulated Kepone when fed live mysids that had grazed on Kepone-laden brine shrimp. Mysids which consumed brine shrimp with Kepone residues of 0.05 or 2.33 ppm attained whole-body Kepone residues of 0.023 (estimated) or 1.23 ppm, respectively, within 72 hours. Kepone residues in the spot approached the concentration of their food; at the lower concentration, Kepone residues that were below detection in the living food organisms accumulated in the predator to detectable concentrations of 0.02 ppm within 30 days.

The maximum overall accumulation and transfer of Kepone or food-chain potential from water to algae and finally to oysters was 2.1, but the transfer potential (i.e., the transfer of Kepone from one trophic level to the next) from algae to oysters was only 0.007. These data indicate that transfer and retention of Kepone from algae by oysters were inefficient. Conversely, the food-chain potential from water to brine shrimp to mysids and finally to fish ranged from 3.9 to 10.5. The transfer potential from brine shrimp to mysids was 0.53 and from mysids to spot was 0.85 indicating that much of the Kepone was being transferred through these trophic levels (Bahner, et al., 1976). The efficiency of Kepone transfer to spot suggests a great potential for Kepone transfer to humans via the consumption of spot.

5.3 TERRESTRIAL BIOTA

5.3.1 Terrestrial Invertebrates

5.3.1.1 Mirex--

The Mirex Advisory Committee (1972) reported that mirex residue levels are extremely low (0.05 ppm) or nondetectable in phytophagous animals selected from mirex-treated areas. Most terrestrial invertebrates have been found to contain less than 0.1 ppm but general scavengers such as crickets and cockroaches, which may consume mirex bait directly, have

TABLE 5.5. BIOCONCENTRATION FACTORS FOR SELECTED SPECIES EXPOSED TO MEASURED CONCENTRATIONS OF KEPONE IN WATER[a]

Species	Concentration, ppb	Length of Exposure	Mean Bioconcentration Factor
Chlorococcum sp., alga	100 (static)	24 hours	340
Crassostrea virginica, oyster	0.03	19 days	9,354
Crassostrea virginica, oyster	0.39	21 days	9,278
Artemia salina, brine shrimp	5.00 (static)	48 hours	10
Artemia salina, brine shrimp	100 (static)	48 hours	23
Mysidopsis bahia, mysid	0.026	21 days	5,962
Mysidopsis bahia, mysid	0.41	21 days	13,473
Palaemonetes pugio, grass shrimp	0.023	28 days	5,127
Palaemonetes pugio, grass shrimp	0.4	28 days	11,425
Cyprinodon variegatus, sheepshead minnow	0.05	28 days	7,115
Leiostomus xanthurus, spot	0.029	30 days	3,217
Leiostomus xanthurus, spot	0.4	30 days	2,340
Leiostomus xanthurus, spot	1.5	96 hours	1,120
Leiostomus xanthurus, spot	3.4	96 hours	941
Leiostomus xanthurus, spot	4.4	96 hours	1,591
Leiostomus xanthurus, spot	12.0	96 hours	900
Leiostomus xanthurus, spot	16.0	96 hours	1,050

[a] Adapted from Bahner, et al., (1976).

been reported to contain mirex residues ranging from 10 to 30 ppm. Predaceous arthropods such as spiders have been found to contain residues higher than those in general invertebrate populations. Spiders may contain in excess of 1.0 ppm mirex 1 year after treatment. Although a decline in populations has been reported for crickets, oil-loving ants and ground beetles following mirex bait application, there is no evidence available to indicate that these populations are permanently affected (Mirex Advisory Committee, 1972).

Lee (1974) reported the results of an investigation in which mirex was experimentally applied to oak and dogwood leaf litter. Two forms were used, corncob grit bait and spray--at the normal application rate of 1.7 g mirex per acre and at ten times the normal rate to evaluate its effects on ecological processes. Ants were essentially removed from bait and concentrated spray areas up to 79 days after treatment; centipedes were decreased on these same areas. The low spray treatment did not affect ants. Spiders, millipedes, beetles, and scorpions were not affected. No patterns of change in microarthropod populations were detected. Decomposition of leaf litter was accelerated in all treatments when compared to control areas. The author speculated that mirex may have accelerated decomposition through its ability to induce mixed-function oxidase systems in bacteria and fungi. These enzymes are responsible for the breakdown of some xenobiotics. The synthesis of these enzymes may have allowed faster metabolism of the plant inhibitor tannic acid, resulting in an early boost in the decomposition process.

Atkins, et al., (1975) classify mirex as moderately toxic; it can be used around bees if dosage, timing and method of application are correct, but should not be applied directly to bees in the field or at the colonies. Mirex has an LD_{50} of approximately 7.15 microgram/bee. Extrapolating on the basis of the dose mortality curves presented, mirex could be applied at a rate of up to 3.9 kg/ha (3.5 lb/ac) without causing over 20 percent mortality. Similarly, a dosage of 12 kg/ha (10.7 lb/ac) would result in approximately 70 percent mortality. Normal application rates of mirex (4.2 g/ha, 1.7 g/ac) represent less than 0.2 percent of the 3.5 lb/acre dosage needed to cause 20 percent mortality.

5.3.1.2 Kepone--

Atkins and Anderson (1962) present evidence that Kepone does not pose a significant threat to honeybees and predaceous insects. Their studies indicated that bees from hives exposed regularly to DDT over a period of 10 years developed a resistance to DDT and, moreover, this resistance was carried over to a number of other chlorinated insecticides, including Kepone. A later publication by Atkins, et al., (1973) calculated that to cause a 50 percent decrease in honeybee population, Kepone would have to be applied at 11.65 kg/ha (10.4 lb/ac). This dosage amounts to about 5 times the application rate recommended by the U.S. Environmental Protection Agency (1976) to control the banana root borer and 10 times that recommended for control of tobaco wireworms. At the 2.25 kg/ha (2 lb/ac) level of application, Kepone was not harmful to three out of four predaceous insects and arthropods monitored in an apple orchard.

5.3.2 Amphibians and Reptiles

Extremely little information is available on the toxicity or metabolism of mirex in amphibians and reptiles. The only available data come from environmental surveys which monitored mirex accumulation in non-target organisms following mirex bait application.

Collins, et al., (1974) sampled 61 species of vertebrates following a single application of mirex bait. They concluded that reptiles and amphibians generally accumulated lower residues (0.001-0.828 ppm) than did birds and mammals. Surprisingly, carnivorous reptiles such as water snakes, lizards and skunks did not appear to concentrate mirex as did birds with similar feeding habits. The authors felt that this may be related to the greater relative volume of food consumed by birds as compared with the volume consumed by reptiles.

Similar data on Kepone accumulation in amphibians and reptiles are not available.

5.3.3 Birds

5.3.3.1 Mirex--

5.3.3.1.1 Acute toxicity--Compared to other species, birds are not extremely sensitive to mirex. The LD_{50} and LC_{50} values shown in Table 5.6 are considerably higher than those for mammals and fish. Naber and Ware (1965) found that a daily consumption of 300 to 600 ppm of mirex was necessary to produce weight loss in hens and a significant decrease in egg hatchability and survival of chicks.

5.3.3.1.2 Subacute toxicity--The U.S. Department of the Interior Fish and Wildlife Service conducted tests to determine lethal dietary toxicities of mirex to bobwhite, Japanese quail, ring-necked pheasant, and mallards (Hill, et al., 1975). The LC_{50} as used in this procedure is ppm toxicant (based on active ingredient) in an *ad libitum* diet producing 50 percent mortality in 8 days (5 days of toxic diet followed by 3 days of untreated diet). Up to six different dietary concentrations of the pesticide were used in each test and LC_{50} values based upon these concentrations were determined by means of computerized probit analysis (Table 5.7). These values seem consistent with those of Table 5.6. In addition, the toxicity of dieldrin relative to that of mirex (expressed as the RTD) is one of the highest among 131 pesticide compounds tested in this study, confirming the findings of rather low acute toxicity of mirex to birds. Similar subacute toxicity studies by Baetcke, et al., (1972) indicated that 8 to 81 percent mortality occurred in quail, mallards, pheasants and cowbirds fed diets containing 200 to 500 ppm mirex for 30 to 111 days.

TABLE 5.6. REPRESENTATIVE ORAL LD_{50}
VALUES FOR MIREX[a]

Species	Exposure route	LD_{50} mg/kg	LC_{50} ppm
Mallard	Oral	2,400	
Coturnix	Oral		10,000
Pheasant	Oral		1,400 to 1,600

[a] Source: Waters (1976).

TABLE 5.7. DIETARY TOXICITIES OF MIREX TESTED IN 5-DAY DIETS OF YOUNG BOBWHITES, JAPANESE QUAIL, RING-NECKED PHEASANTS, AND MALLARDS[a]

Species	Age,[b] days	Number of Concen-trations[c]	Toxicity Statistics			
			LC_{50}[d]	95% Confidence Interval	RTD[e,f]	95% Confidence Interval
Bobwhite	14	6	2511	2160-2908	60.6	49.3 -74.2
Japanese quail	14	1	>5000	20% mortality		
Ring-necked pheasant	14	6	1540	1320-1789	29.3	24.1 -37.7
Mallard	10	3	>5000	No mortality		

[a] Adapted from Hill, et al., (1975).

[b] Age of birds at start of test.

[c] Number of dietary concentrations used in probit analysis.

[d] LC_{50}: ppm compound (based on active ingredient) in ad libitum diet calculated to produce 50 percent mortality in 8 days (5 days of toxic diet followed by 3 of untreated diet).

[e] Relative toxicity of dieldrin (RTD) read as: "Dieldrin is x times as toxic as the given compound as tested".

[f] Dieldrin toxicity statistics are mean values for all comparable dieldrin tests (sample size: bobwhite, 7; Japanese quail, 15; ring-necked pheasant, 19; 10-day-old mallard, 11; and, 5-day-old mallard, 6).

Waters (1976) examined the distribution pattern of mirex in laying hens fed 1.06 ppm mirex in the diet. Mirex appeared to be readily absorbed from the digestive tract and distributed throughout the body. At 27 weeks, highest levels were found in fat, 15.15 ppm; other organ levels were kidney, 2.16 ppm; liver, 0.49 ppm; and breast, 0.11 ppm. Levels in all tissues were elevated after 39 weeks of treatment: fat, 24.8 ppm (~64 percent increase); kidney, 3.4 ppm (~59 percent increase); liver, 1.9 ppm (~294 percent increase); and breast, 0.31 ppm (~182 percent increase).

5.3.3.1.3 Metabolism--Ivie, et al., (1974b) studied accumulation, distribution and excretion of ^{14}C-mirex in quail exposed to up to 30 ppm mirex in the diet. After 16 months of continuous mirex treatment, radiocarbon residues in the fat of male quail were almost 200 times higher than dietary concentrations and showed little tendency toward reaching a plateau of residue accumulation. Skin consistently showed high radiocarbon concentrations also. As shown in Table 5.8, other tissues analyzed revealed much lower radiocarbon than did fat. Male and female quail exhibited differences in residue patterns. Once the females began producing eggs (at about 6 weeks of age), residues in all their tissues were substantially lower than in males. In contrast to males, females rapidly established an equilibrium between mirex intake and elimination through the eggs, which resulted in a plateau in all tissues. Female quail were also considerably more efficient at eliminating existing body burdens of radiocarbon following cessation of the ^{14}C-mirex diet. After 3 months, residual radiocarbon in most tissues was reduced by at least 75 percent. Male quail excreted at least 40 percent of the ^{14}C-mirex in a comparable period. Numerous other studies have documented the importance of egg-laying as a means of eliminating body burden in female birds (Ivie, et al., 1974b; Waters, 1976; Foster, 1974).

5.3.3.1.4 Reproductive effects--Significant effects on reproduction were not seen in bobwhite quail fed 10 to 40 ppm mirex and mallards fed 1 to 10 ppm. Some suppression of egg production occurred in the mallards but none occurred in the bobwhites. Rapid transmittal of mirex to eggs in treated birds and a significant accumulation of mirex residues in brains and carcasses occurred at these dosages (Woodham, et al., 1975). Levels up to 200 ppm of mirex appeared to be tolerated in laying hens without adverse effects on various reproductive parameters such as egg hatchability and chick growth and survival (Waters, 1976). In a similar study by Hyde, et al., (1973b), mallard ducks fed dosages of 1 and 100 ppm of mirex did not differ significantly from controls on egg production, shell thickness, shell weight, embryonation and hatchability. Duckling survival was significantly reduced (P 0.01) in the group fed 100 ppm, however.

Eggshell abnormalities such as thinning in some instances and thickening in others were a result of dietary mirex exposure (Waters ,1976). The mechanisms by which organochlorines affect eggshell thickness have not been elucidated but may include (a) stimulation of hepatic microsomal enzymes which degrade steroids necessay for calcification, (b)

TABLE 5.8. ^{14}C RESIDUES IN THE TISSUES OF JAPANESE QUAIL FED 30 PPM OF MIREX-^{14}C IN THE DIET[a]

	Ppm of Mirex Equivalents											
	Brain		Muscle		Liver		Kidney		Skin		Feathers	
Time	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
	Birds on treatment											
4 days[b]	1.6		4.9		34.3		7.1		48.8		5.9	
7 days	2.5		5.9		47.4		9.4		62.6		14.7	
14 days	2.2	2.4	5.6	5.4	16.7	23.5	12.1	12.1	137.7	180.7	15.5	8.8
1 month	3.3	3.5	8.0	12.4	30.2	30.5	21.1	13.3	233.2	130.4	25.7	30.4
6 months	4.9	1.4	50.6	10.0	55.1	55.7	58.2	18.3	600.5	92.8	36.2	26.5
12 months	7.4	1.9	72.8	7.9	114.4	25.5	64.0	10.4	1495.4	99.1	196.7	39.6
16 months	7.2	2.2	87.0	11.4	138.0	30.9	88.9	11.6	872.8	129.0	114.8	33.0
	Removed from treatment after 6 months											
7 days	5.0	2.3	45.8	6.8	62.4	18.2	52.5	5.9	906.1	100.9	47.4	15.8
14 days	5.1	3.1	38.2	5.9	52.3	19.3	61.0	4.6	1028.3	77.9	51.7	8.8
1 month	5.2	1.7	45.2	4.5	51.2	7.1	42.4	2.5	818.2	81.1	40.9	14.3
3 months	0.4	0.5	14.4	1.4	8.6	1.5	4.9	0.6	765.3	40.7	33.5	7.3
6 months	0.2	0.2	3.5	0.5	6.5	0.5	3.5	0.2	620.1	19.5	29.4	5.6
10 months	0.1	0.1	5.0	0.1	5.4	0.1	2.9	0.1	575.8	1.4	28.4	1.9

[a] Source: Ivie, et al (1974). Reprinted, with permission, from J. Ag and Food Chem. (c) American Chemical Society (1974).

[b] Sex of quail could not be determined at this age.

inhibition of medullary bone deposition, (c) inhibition of the parathyroid gland, (d) inhibition of the thyroid, (e) inhibition of calcium from the gut, (f) inhibition of carbonic anhydrase activity in the avian shell-forming gland, and (g) abnormal stimulation of the of the nervous system which results in premature ejection of eggs (Foster, 1974).

5.3.3.1.5 Residue accumulations in wild birds--Numerous wildlife sampling surveys have indicated that birds, as a group, contain relatively high levels of mirex. Most insect-eating birds collected in or near a treated area contained mirex residues greater than 1 ppm, occasionally containing as much as 10 ppm or more. Egg samples from snowy egrets averaged around 13 ppm. The reproductive consequences of mirex residues of this magnitude in eggs are not known. A survey of birds collected from the eastern United States showed mirex residues present only in those from South Carolina, Georgia, and Florida; in a sample of eight birds, an average level of 4.32 ppm (lipid weight) was detected. In starlings, levels of 0.1 to 1.66 ppm mirex have been reported by Waters (1976).

Birds were also sampled in a survey of mirex residue accumulation following a single aerial application of mirex bait (Collins, et al., 1974). Birds accumulated mirex residues at levels in the range of 1 to 8 ppm. The highest concentrations of mirex were detected in loggerhead shrikes and mockingbirds 3 months after treatment. The authors believe that these residues reflect the diet of these birds. Interestingly, green herons did not accumulate high levels of residues as would be expected by virtue of their position in the food chain. An extensive home range of the herons could account for the relatively low residue levels if they derived a large part of their diet from outside the treated area. Birds whose diets consist primarily of seeds, fruits and vegetable matter, such as cardinals, Eastern cowbirds and quail, had lower levels of mirex than the more carnivorous species such as shrikes, meadowlarks and mockingbirds.

Mirex is a very stable compound chemically and residues in certain species are remarkably persistent. For example, adult snowy egrets contained up to 0.64 ppm and nestlings analyzed had up to 3.5 ppm mirex 1 year after treatment with mirex. Insectivorous land birds have been reported to contain from 0.22 to 9.1 ppm mirex 6 to 12 months after treatment (Mirex Advisory Committee, 1972).

A very recent study by Kendall et al., (1977) reported on mirex residues in wild birds. Mirex was aerially applied in early fall to a state game management area in Hampton County, South Carolina, at the rate of 1.7 g mirex per acre (in 1.25 lb corncob grit/soybean oil bait). The area was managed for bobwhite quail hunting and had a severe fire ant infestation.The authors wanted to measure mirex residues in bobwhite available for human consumption because the birds' food habits allow frequent contact with the pesticide. Fat and breast muscle tissues were obtained from birds collected prior to and periodically for nine months following treatment. Pre-treatment samples contained some residues as a result of a mirex treatment on adjacent land. Post-treatment samples had up to a fivefold increase in fatty tissue two weeks after the application; levels averaged 0.23 ppm and 5.1 ppm in breast and fat, respectively (dry weight). Grasshoppers, which have been known to have up to 0.7 ppm mirex in treated areas, were a major component of the bobwhites' diet. Levels in tissues declined during the winter but remained above pre-treatment levels. A sharp peak occurred in the spring, corresponding with insect emergence. Levels in fat remained elevated during the summer following treatment.

Residues in many of the breast muscle tissues and in all the fat tissues appear to exceed the EPA guideline of 0.1 ppm (lipid weight) mirex in meat for human consumption. However, the authors conclude that, in light of the low lipid weight of bobwhite and the reporting of residues on a dry weight basis, the residues would contaminate little, if any, of the human food chain.

Data recently obtained from Canadian researchers (Table 5.9) have indicated the presence of mirex and other persistent toxic substances in herring gull eggs from Lakes Superior, Huron, Erie and Ontario (Alexander, 1976 personal communication). Unlike the previous examples in which the source of mirex residues was prior mirex bait application, in the case of Lake Ontario birds, the source is industrial discharges of mirex which, through uptake by fish, provide a dietary source of mirex for the gulls. Because they eat large quantities of fish which have lipid-soluble chemicals such as mirex, herring gulls are very sensitive indicators of contaminants. While significantly below the levels of Lake Ontario gull eggs, the detectable concentrations of mirex in herring gull eggs from the three other Great Lakes does signify widespread distribution of mirex in this area.

5.3.3.2 Kepone--

5.3.3.2.1 Acute toxicity--Kepone does not appear to be very toxic to birds as an acute poison. Tucker and Crabtree (1970) reported a single dose LD$_{50}$ of greater than 2400 mg/kg of body weight in a study of 3 to 12-month-old mallard ducks. DeWitt et al., (1962) determined a dietary LC$_{50}$ value of 400 ppm for young mallards, 600 and 530 ppm for young and adult bobwhite quail and 600 and 115 ppm for young and adult ringnecked pheasants. All 7 to 14-day chicks fed 200 ppm Kepone exhibited severe tremor after 10 days. Japanese quail injected daily with 0.5 mg Kepone for 10 days exhibited damage to hepatic parenchymal cells (including disruption of mitochrondria), cellular debris in bile and canaliculi. Also, a significant increase in phagocytic kupffer cells lining the liver sinusoids was observed (U.S. Environmental Protection Agency, 1975).

Eroschenko and Wilson (1975) conducted a histological comparison study of reproductive organs, livers and adrenal glands from control immature and adult Japanese quail of both sexes and from quail fed 200 ppm of Kepone under two photoperiod regimes. Kepone had an estrogenic effect on the oviducts of immature females and on the testes of immature

TABLE 5.9. MIREX CONTENT OF HERRING GULL EGGS COLLECTED IN THE GREAT LAKES IN 1974 AND 1975[a]

Location	Year	Number of Samples	Mirex Concentration, ppm	
			Mean[b]	Range[c]
Lake Superior	1974	19	0.80	0.30- 2.56
	1975	20	0.16	0.20- 5.20
Lake Huron	1974	20	0.82	0.22- 6.82
	1975	20	0.27	0.06- 2.01
Lake Erie	1974	20	0.49	0.15- 2.19
	1975	22	0.29	0.14- 0.73
Lake Ontario	1974	19	6.18	2.15-18.6
	1975	20	4.18	1.95-10.0

[a] Source: Alexander (1976) personal communication.

[b] Geometric means in ppm on wet weight basis.

[c] Range based on 10 egg random samples, 1 per clutch, from 2 colonies in each lake.

and mature adults. Kepone increased cellular proliferation, cytodifferentiation and tubular gland formation in the oviducts of immature quail under both long and short photoperiods. This accelerated development and maturation of the oviduct did not produce any evidence of cellular abnormalities or degeneration. No cellular changes were noted in oviducts of laying (mature) quail. Ovarian tissue from Kepone-treated quail contained more primary occytes and smaller follicles when compared with control birds. In the liver, cells from all of the experimental birds were filled with large, lipid-like inclusions. Likewise, in all Kepone-treated birds, hypertrophy of adrenal cortical and medullary cells was seen. A detrimental effect was also recorded in the testes of both immature and adult quail under a 16 hours of light-8 hours of darkness light regimen. Distended seminiferous tubules contained watery fluid which was apparently responsible for the significant change in testes weight. Since these changes adversely affect spermatogensis, a high percentage of reproductive failure would be anticipated.

5.3.3.2.3 Metabolism--No data on the metabolism of Kepone in birds was identified.

5.3.3.2.4 Reproductive effects--Despite the rather low acute toxicity of Kepone in birds, hazardous effects on reproduction affecting both male and female birds have been consistently reported in a variety of species. Degeneration and abnormality of the testes have been observed in both young and adult males in at least four laboratory studies of Japanese quail. Pathologic findings in the testes reported earlier by Eroschenko and Wilson (1975) would indicate decreased spermatogensis and sperm mobility in quail fed 200 ppm Kepone.

Generally, an "estrogen-like" effect has been observed in both sexes. In females, this effect is manifested by accelerated sexual development of immature female birds, while in the males the effects of Kepone are much more devastating. Male ring-necked pheasants fed Kepone at 50, 100 or 150 ppm developed adult female plumage accompained by abnormal testes, malformed sperm, and a high percentage of reproductive failure (DeWitt, et al., 1962). The exact nature of the "estrogen-like" effect of Kepone is unknown but Mcfarland and Lacy (1969) have proposed a two-fold mechanism. First, Kepone stimulates the hypothalamus to increase follicle-stimulating hormone (FSH). FSH in turn stimulates estrogen secretion and acts directly as an estrogenic agent.

Naber and Ware (1965) concluded that the addition of 75 or 100 ppm of Kepone to the diet of hens results in a significant reduction in egg production. (Mirex on the other hand, did not significantly reduce egg production at either the 300 or 600 ppm feeding levels). The apparent hatchablity of eggs was unaffected, but reduced emryonic survival was observed in the 100 ppm Kepone group. Survival of chicks during a 14- or 20-day period following hatching was also reduced. Only 56 percent of the chicks from hens fed 75 ppm of Kepone survived; at the 100 ppm feeding level, none of the chicks survived. Most of the chicks exhibited a nervous syndrome. Studies of yolk residues showed high insecticide residues still present in eggs 3 weeks following removal of the hens from the treated diet.

Like DDT and other organochlorine pesticides, dietary Kepone can cause teratogenic eggshell deposition. Major defects were noted in the cuticle and vertical crystal layers of the shell when Japanese quail were fed 225 ppm Kepone for 21 days (U.S.EPA, 1975). Erben (1972) reported that Kepone caused formation of a peculiar thick layer of aragonite and vaterite and thickening of the cuticle layer. This increase in the thickness of the spongy layer leads to blockage of shell pores, resulting in suffocation of the embryo.

5.3.3.2.5 Residue accumulations in wild birds--The accumulation of Kepone in wild birds does not appear to have been investigated as yet.

5.3.4 Mammals

5.3.4.1 Comparative Acute Toxicity of Mirex and Kepone--

Gaines (1969) presented data on the acute toxicity (LD_{50}'s) for 98 pesticides administered in a single dose by the oral or dermal route to adult Sherman strain rats. These data for the pesticides Kepone and mirex (in two formulations) are presented in Table 5.10. The compounds were formulated so that the different dosage levels of poison could be given by stomach tube by administering the formulation at a constant rate of 0.005 ml/g body weight. Dermal applications were at a constant rate of 0.008 ml/g body weight. The LD_{50} and LD_1 values and the lowest dose of pesticide to kill a rat are given in Table 5.10. The LD_1 value is probably a more realistic measure of the toxic hazard of a compound than is the LD_{50}, and with the LD_{50} value it indicates the slope of the dose-response line. As can be seen from the table, Kepone has a lower LD_{50} than mirex when administered orally, yet Kepone and mirex have the same dermal toxicity. Male and female rats were equally susceptible to Kepone in oral and dermal administrations; however, mirex is slightly more toxic to female rats.

5.3.4.2 Mirex--

5.3.4.2.1 Acute toxicity--Mirex administered as an acute oral dose is not very toxic. The acute LD_{50} of mirex for rats lies in the range from 365 to 740 mg/kg. Gaines and Kimbrough (1970) reported an acute LD_{50} of 365 mg/kg in female Sherman rats. A somewhat lower LD_{50} of 740 mg/kg in males and 600 mg/kg in females was reported earlier by the same investigator (Gaines, 1969).

5.3.4.2.2 Subacute and chronic toxicity--The most commonly observed subacute toxic effects of mirex in mammals are weight loss, increased liver weight and reproductive difficulties (Gaines and Kimbrough, 1970; Brooks, 1976; Khera, et al., 1976; Mirex Advisory Committee, 1972).

TABLE 5.10. THE ACUTE ORAL AND DERMAL TOXICITY OF KEPONE AND MIREX IN RATS[a]

	Oral, mg/kg						Dermal[b], mg/kg					
	LD_{50}		LD_1		Lowest dose to kill a rat		LD_{50}		LD_1		Lowest dose to kill a rat	
Compound	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Kepone	125	125	92	92	100	125	>2000	>2000	>2000	>2000	>2000	>2000
Mirex	740[c]	600[c]	200[c]	270[c]	400[c]	500[c]	>2000	>2000	>2000	>2000	>2000	>2000
Mirex[d]	>3000	>3000			>3000	>3000						

[a] Source: Gaines (1969). Reprinted, with permission, from Toxicology and Applied Pharmacology. (c) Academic Press, Inc. (1969).

[b] Dosage rate, 0.008 ml/g.

[c] Five percent in corn oil solution.

[d] Twenty percent suspension in corn oil.

Subacute effects were observed in rats at the 5 ppm feeding level (Gaines and Kimbrough, 1970). Rats fed 5 ppm mirex for 166 days exhibited minimal, but definite pathological changes in the liver including slightly enlarged liver cells, vacuolated cytoplasm and occasional inclusions.

At the 25 ppm feeding level, most of the rats had enlarged multi-nucleated liver cells with smooth or vacuolated cytoplasm. Increases in smooth endoplasmic reticulum and free ribosomes, decreased glycogen and osmiophilic dense bodies, and bile stasis were also reported at this dosage. The Mirex Advisory Committee (1972) reported similar findings of enlarged liver and vacuolation of liver cells in rats fed a dietary dosage of 80 ppm mirex. Deaths were observed at 1280 ppm and growth suppression occured at 320 ppm. Dogs fed daily dosages of 0, 4,20 and 100 ppm of mirex showed no effects at the 20 ppm dosage level, but the 100 ppm dose level was lethal.

Chronic exposure to mirex has produced a wide range of effects in laboratory animals. This insecticide is a documented teratogen (Khera, et al., 1976) and produces a number of hepatotoxic and enzymatic changes and reproductive effects (Gaines and Kimbrough, 1970; Byard, et al., 1975; Kendall, 1974; Baker et al., 1972; and Abston and Yarbrough, 1974, 1976). While it is suspected that mirex may be a carcinogen, results of two separate studies have yielded somewhat inconclusive results (Innes, et al., 1969; Ulland, et al., 1973; Ulland, et al., 1977) due to inadequate experimental designs and poor survival rates of the test animals. These effects are more fully discussed in Sections 5.3.4.2.4 through 5.3.4.2.10.

Hayes (1967) has described a technique for calculating a chronicity factor. This means of expressing the results of repeated dosing may be used to compare the tendency of different compounds to have cumulative effects without reference to their absolute toxicities. The chronicity factor is defined as the single dose LD$_{50}$ in mg/kg divided by the 90-dose LD$_{50}$ in mg/kg/day. The 90-dose oral LD$_{50}$ of mirex was found to be 6 mg/kg/day which yields a chronicity factor of 60.8 (Mirex Advisory Committee, 1972). This chronicity factor is by far the highest observed for any pesticide to date. Comparable chronicity factors for DDT and dieldrin are less than 5.6 and 12.8, respectively. Thus, mirex is highly cumulative in effect; and like many other chlorinated hydrocarbons, it is probably metabolized or excreted very slowly (Gaines and Kimbrough, 1970).

5.3.4.2.3 Metabolism--Ivie, et al., (1974b) studied accumulation, tissue distribution, and excretion of ^{14}C-mirex in rats during extended dietary exposure. Dosages administered to the treated rats were 0.3, 3.0, and 30.0 ppm of mirex mixed into commercial rat meal. Uptake and retention of radiocarbon in fat occurred rapidly after ingestion of the treated food. As shown in Table 5.11, a steady pattern of accumulation is seen in the organs. In rats maintained on the mirex diet for 16 months, mirex concentrations in the fat were approxiately 120-fold higher than corresponding dietary intake levels. No plateau of residue accumulation was observed in any tissue during the feeding period. Remarkably, the

TABLE 5.11. RADIOACTIVE RESIDUES IN THE TISSUES OF RATS FED 30 PPM OF MIREX IN THE DIET[a]

Time	Ppm of mirex equivalents[b]							
	Brain	Muscle	Liver	Kidney	Skin	Subcutaneous Tissue[c]	Hair	Fat[d]
				Rats on Treatment				
7 days	5.5	5.5	15.7	9.6	25.7	33.4	6.1	81
14 days	6.6	50.0	32.0	11.1	40.9	138.7	9.7	--
1 month	8.6	6.4	38.9	12.7	82.4	170.5	5.4	332
6 months	18.1	9.1	63.4	22.1	229.6	211.3	11.8	2047
12 months	23.9	26.5	98.9	34.3	374.5	278.7	22.6	2808
15 months	36.9	49.5	158.0	52.1	421.6	321.3	25.2	3730[e]
				Rats Removed from Treatment after 6 Months				
7 days	15.7	8.6	61.2	18.0	231.3	204.6	6.7	1980
14 days	14.2	8.6	58.6	16.9	220.9	189.0	7.3	1907
1 month	12.6	7.4	55.5	14.9	216.7	198.2	6.5	1670
3 months	11.0	6.6	42.8	12.5	176.2	40.7	5.3	1490
6 months	10.3	6.4	32.8	10.8	184.4	67.2	5.4	1440
12 months	9.9	6.3	25.2	10.1	133.6	55.7	4.9	1340

[a] Source: Ivie, et al (1974). Reprinted, with permission, from J. Ag and Food Chemistry. (c) American Chemical Society (1974).

[b] Ratios of the levels of residues in the tissues to those in the diet were the same for all three feeding levels, 0.3, 3.0, and 30.0 ppm.

[c] Connective tissue between epidermis and muscle.

[d] Females only.

[e] Based on 16 months.

ratios of the levels of residues in tissues to those in the diet were essentially identical for all three feeding groups. For example, the 3.0 ppm group accumulated ten times the residues of the 0.3 ppm group and one-tenth that of the 30 ppm feeding group. Radiocarbon dissipated very slowly from the tissues following cessation of the treated diet; one-half or less of the residues had been eliminated 10 months after treatment stopped. A majority of the total mirex consumed was retained by the body tissues. Radioassay of feces and urine throughout the study indicated that approximately 25 percent of the total ingested radiocarbon was eliminated by the fecal route; excretion via the urine was negligible. GC and TLC of radiocarbon residues from feces and kidney samples revealed only a single compound which corresponded in chromatographic behavior to mirex. Analysis by mass spectrometry confirmed that the residue was indeed the unmetabolized parent compound. Hundreds of analyses over the 16-month period of this investigation showed no evidence of mirex metabolism. In addition, the levels of mirex residues accumulated in tissues were considerably higher than those reported for other lipophilic pesticides. Furthermore, following cessation of treatment, body burdens of mirex in rats dissipated at a much slower rate than those of either DDT or dieldrin. In spite of the relatively poor absorption of mirex from the gut, the extraordinary long half-life in the tissues indicates the potential for accumulation of high levels of mirex even at low doses. Also, since mirex is not degraded by either plants or animals, accumulated mirex can be expected to be recycled upon the death of the organism. Ivie, et al., (1974b) concluded from these and earlier studies that mirex is probably the most stable organic pesticide known.

A study by Mehendale, et al., (1972) was undertaken to determine the fate of a single dose of mirex such as might be encountered by an animal in the environment. Their report identifies the routes and rates of excretion and the amounts and locations of storage in the bodies of a group of male rats fed a single dose of 6.0 mg/kg of ^{14}C-mirex. Findings were (1) the primary route of mirex excretion is the feces; (2) 55 percent of the administered dose of mirex was excreted in the first 48 hours after administration, most probably representing that portion of the dose which passed unabsorbed through the gut. (3) total excretion levels off rapidly after 48 hours and very little is excreted in the urine, suggesting that once absorbed mirex is readily stored and only slowly excreted. Excretion kinetics appear to be biphasic; the initial "fast" phase lasts about 38 hours and the slow phase (second half-life) is estimated to be in excess of 100 days. (4) Urinary excretion accounted for less than 1 percent of excretion and analysis did not reveal any radioactive compound other than mirex; (5) within the body, tissues and organs retained approxmately 34 percent of the total dose at necropsy 7 days after administration of the dose. Out of this 34.2 percent retained, the tissue distribution of the stored mirex was approximately as follows: 27.8 percent in fat, 3.2 percent in muscle, 1.75 percent in liver, 0.76 percent in kidney, and 0.23 percent in the intestines. Table 5.12 shows the distribution of ^{14}C-mirex in the rats. No radioactive compound other than mirex was detected.

TABLE 5.12. DISTRIBUTION OF ^{14}C-MIREX ADMINISTERED ORALLY TO RATS[a,b]

Tissues or Organs	Percent of Total Dose Retained[c] per g of Tissue[c]	Percent of Total Dose Retained[c] Total
Fat	1.54	27.8
Liver	0.15	1.75
Small intestine	0.10	0.76
Muscle	0.04	3.20
Large intestine	0.10	0.23
Stomach	0.05	0.06
Heart	0.05	0.03
Kidney	0.25	0.09
Brain	0.04	0.07
Testes	0.06	0.12
Lung	0.07	0.08
Subtotal		34.19
Excretion		
Feces		58.5
Urine		0.69
Total recovery		93.38

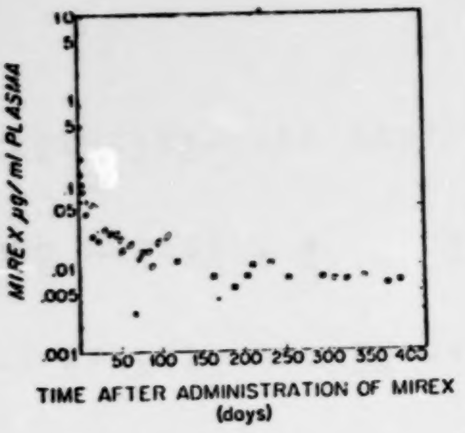
[a] Source: Mehendale, et al. (1972). Reprinted, with permission, from Bull. Environmental Contamination and Toxicology. (c) Springer-Verlag New York Inc. (1972).

[b] 6.0 mg/kg mirex ^{14}C administered by oral intubation.

[c] Wet weight basis.

A similar investigation by Gibson, et al., (1972) examined the fate of mirex along with a major photoproduct in rats. In general, the results of this investigation were quite similar to those of the study by Mehendale, et al., (1972) mentioned above. Rats receiving single doses of ^{14}C-mirex eliminated about 18 percent of the administered radiocarbon during a 7-day period. Eighty-five percent of this amount was eliminated via the feces within 48 hours after administration. Only trace amounts of radioactivity were detected in the urine. Essentially all of the excreted radioactivity was unmetabolized mirex. Intestinal absorption of mirex was slightly depressed by the presence of an existing body burden. Mirex demonstrated an affinity for lipids; and once absorbed into fatty tissue, the residue levels remained essentially unchanged for periods up to 28 days. TLC and GC analyses revealed that the radiocarbon residues in fat consisted entirely of unaltered mirex. The photoproduct was also highly lipophilic in nature and chromatographic analysis revealed that the photoproduct radiocarbon stored in fat 7 days posttreatment was also entirely unmetabolized.

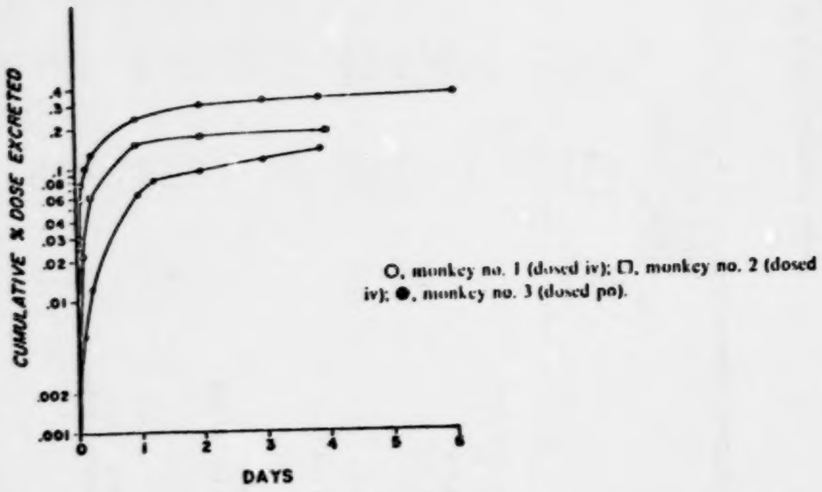
Two recent articles describe mirex kinetics in the rhesus monkey and develop a pharmacokinetic model of mirex disposition in the body (Wiener, et al., 1976; Pittman, et al., 1976). The first study administered ^{14}C-mirex to three female rhesus monkeys in a single dose equivalent to about 1 mg/kg either intravenously (two animals) or orally (one animal). Blood plasma, urine, feces, and tissue samples were obtained periodically and analyzed for ^{14}C-mirex. Tissues were obtained at autopsy at 23, 106, and 388 days after dosing for the three monkeys, respectively. Absorption patterns varied according to the method of administration, with the intravenous doses producing initial high levels which declined rapidly over the next few hours. Oral dosage produced measurable ^{14}C-mirex in the plasma 2 hours after administration, reaching a maximum at 5 hours. Thereafter, the rate of decline in plasma radioactivity was similar in all three monkeys; and within 24 hours, plasma radioactivity was less than 5 percent of the earliest value measured. Plasma ^{14}C-mirex continued to decline at a much slower rate and remained in measurable quantities during the study. Urinary excretion declined rapidly over the first week with total urinary excretion over this period amounting to less than 0.6 percent of the dose. Figures 5.3 and 5.4 show plasma and urinary levels as a function of time since dosage. In all tissues analyzed, ^{14}C-mirex was detected. Fat had by far the highest concentration, followed by adrenal gland, peripheral nerves, thyroid gland and skin. Tables 5.13 and 5.14 show tissue content and percent of administered mirex found in tissues at autopsy. Excretion of ^{14}C-mirex in feces was initially rapid; about 25 percent of the dose appeared in the feces of the orally-dosed monkey within 48 hours. Daily excretion of mirex in the feces of intravenously-dosed monkeys closely paralleled mirex levels in plasma. Cumulative fecal excretion of ^{14}C-mirex over a period of 1 year was less than 7 percent of the dose. No metabolites of mirex were isolated after 2 days in fecal samples; however, extracts of fecal samples after 14 days or more showed small amounts (approximately 3 percent of the total ^{14}C-content) of an unidentified compound more polar than mirex. The authors suggested the possibility that this unidentified substance found in the



O, monkey no. 1 (dosed iv); □, monkey no. 2 (dosed iv); ●, monkey no. 3 (dosed po).

FIGURE 5.3. Plasma levels of mirex after administration of ^{14}C-mirex to female rhesus monkeys.

Source: Wiener, et al. (1976). Reprinted, with permission, from Drug Metabolism and Disposition. (c) American Society for Pharmacology and Experimental Therapeutics (1976).



O, monkey no. 1 (dosed iv); □, monkey no. 2 (dosed iv); ●, monkey no. 3 (dosed po).

FIGURE 5.4. Cumulative urinary excretion of ^{14}C after administration of labeled mirex to female rhesus monkeys.

Source: Wiener, et al. (1976). Reprinted, with permission, from Drug Metabolism and Disposition. (c) American Society for Pharmacology and Experimental Therapeutics (1976).

TABLE 5.13. TISSUE CONTENT OF RADIOACTIVITY AS MIREX IN FEMALE RHESUS MONKEYS AFTER ADMINISTRATION OF A SINGLE DOSE OF ^{14}C-MIREX[a]

	Mirex Equivalents, ng/g of Tissue				Mirex Equivalents, ng/g of Tissue		
	No. 3, 23 Days (Oral)	No. 1, 106 Days (iv)	No. 2, 388 Days (iv)		No. 3, 23 Days (Oral)	No. 1, 106 Days (iv)	No. 2, 388 Days (iv)
Fat				Other			
Bone marrow (yellow)	1200		2140	Bile[b]	40	120	12
Brown		4960		Plasma[b]	23	19	7
Lipoma of brow			1250	Aorta	36		61
Mesenteric	2570		2070	Bladder	238		28
Omental	2120	6930	2950	Diaphragm	183		30
Peri-renal		5760	1860	Eyeball	11		
Subcutaneous	1680	5880	2630	Fallopian tubes	61	156	20
				Hair	81		38
Endocrine System				Heart (ventricles)	75	61	47
Adrenal	541	2630	651	Intestine			
Ovaries				Small	100	103	29
Corpus luteum			100	Large	743	349	105
Interstitial gland	302	262	66	Kidney	65	61	43
Pancreas	137	112	139	Liver	341	363	132
Pituitary	35	110	15	Lung	36	61	43
Thyroid	93	865	168	Muscle, skeletal	39	34	10
				Parotid	64		53
Nervous System				Skin		1017	251
Cerebrum-frontal lobes	102	81	27	Spleen	32	34	14
Cerebellum	83	79	31	Stomach	61	105	28
Medulla	134	110	47	Tongue			38
Meninges	18		11	Uterus			
Peripheral nerve				Myometrium	16	20	7
Sciatic	268	874	332	Endometrium (proliferative)			13
Brachial plexus			350	Vena cava	181		161
Pons		130					
Spinal cord	102	143	55				
Thalamus		85					

[a] Source: Wiener, et al. (1976). Reprinted, with permission, from Drug Metabolism and Disposition. (c) American Society for Pharmacology and Experimental Therapeutics (1976).

[b] mg/ml.

TABLE 5.14. PERCENT OF ADMINISTERED MIREX FOUND IN VARIOUS TISSUES AT AUTOPSY OF FEMALE RHESUS MONKEYS[a]

Tissue	Percentage of Dose No. 3, 23 Days	No. 1, 106 Days	No. 2, 388 Days
Fat[b]	55.3	87.4	85.7
Skin[c]		10	3.7
Skeletal Muscle[d]	2.0	1.7	0.60
Large Intestine	1.01	0.36	0.21
Liver	0.92	0.51	0.42
Brain	0.22	0.13	0.05
Small Intestine	0.14	0.11	0.06
Stomach	0.049	0.051	0.019
Heart	0.035	0.017	0.022
Lungs	0.026	0.027	0.032
Pancreas	0.039	0.011	0.015
Kidney	0.025	0.017	0.012
Adrenal	0.0055	0.033	0.012
All other tissues analyzed[e]	0.016	0.016	0.0097
Total measured in urine	0.64	0.37	0.18
Total measured in feces	26.53	4.69	6.91
	87.0	105.4	98.0

[a] Source: Wiener, et al. (1976). Reprinted, with permission, from Drug Metabolism and Disposition. (c) American Society for Pharmacology and Experimental Therapeutics (1976).

[b] The total fat on monkey No. 1 was estimated to be 15 percent of body weight. Monkeys No. 2 and No. 3 were obese and the estimate was 25 percent of body weight.

[c] Skin was estimated to be 10 percent of body weight.

[d] Skeletal muscle was estimated to be 50 percent of body weight.

[e] None of the remaining tissues analysed contained more than 0.0089 percent of the dose.

feces might have been a spontaneous decomposition product, resulting from prolonged exposure to fluorescent light in the cage (photodecomposition) rather than bacterial decomposition in the gut of the monkey, since collection and analysis were delayed.

In a related study, Pittman, et al., (1976) measured ^{14}C-mirex in plasma, urine and feces at intervals after dosing and in tissues at autopsy. Graphical analysis of plots of the logarithm of plasma concentration vs time provided estimates of the values of the first-order rate constants needed for the pharmacokinetic models. These parameter values are shown in Table 5.15. A computer program, FITKIN, was used to solve the differential equations for each model and to adjust these numerical solutions to obtain a normalized, least-squares fit. A mammillary, four-compartment, open system model was selected from among the alternatives since this model allowed for urinary excretion of mirex from a "central" compartment and for fecal excretion of mirex from a "fast" tissue compartment. This model yielded theoretical data which were in good agreement with the observed values. A schematic diagram of the model is shown in Figure 5.5.

Figure 5.6 shows the plasma level vs time for monkeys 1 and 2 as a function of days after administration. For the first monkey, the last few plasma concentration data points were omitted because of an accidental failure to feed this monkey for a few days, as indicated by the arrow in Figure 5.6. Apparently the starvation period resulted in fairly rapid release of depot mirex into the central compartment.

Figure 5.7 shows the cumulative fecal and urinary excretion of mirex as a function of days after administration. At the time each monkey was killed, the predicted dose remaining in the fourth compartment (the "very slow" tissue compartment) was 87 percent for Monkey No. 1 and 84 percent for Monkey No. 2. These values are in good agreement with the percentage of the dose estimated to be present in the fat at autopsy, 86 and 87 percent, respectively. Principal findings of the study were: (1) The fit of the data for fecal and urinary excretion with the model was acceptable, indicating that mirex distribution is not influenced by the route of administration, save that the differences in initial absorption must be accounted for. (2) The maximum concentration of mirex in the "very slow" compartment did not occur until 3 months to a year after a single dose. (3) Even though excretion of mirex in urine and feces was continuous, the projection estimated a decrease in the body burden of only 1-2 percent of the amount present at the end of the first year over the next 4 years, as shown in Figure 5.8. The values shown were calculated using computer-optimized parameters. (4) The program MODEL was used to simulate the disposition of mirex during hypothetical dietary regimens of 0.25 and 1.0 mg/kg/day for 10 years. For the first 3-6 months of the simulation, the rate at which the projected quantity of mirex in any compartment increased varied noticeably, the rate of change decreasing in the central compartmment and increasing in the peripheral compartments. After 6 months, the rate of increase in all compartments appeared to be linear; plateau levels were not reached in 10 years.

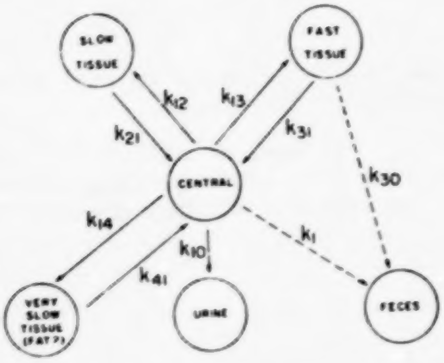
TABLE 5.15. PARAMETERS FOR MIREX MODEL IN MONKEYS[a]

Parameter[b]	Values in Monkey No.		
	1	2	3
k_{12}	0.4582	0.1938	0.4508
k_{21}	0.05607	0.02530	0.0608
k_{13}	0.7935	1.013	0.9909
k_{31}	0.6372	0.3157	0.4817
k_{14}	0.3451	0.1881	0.2890
k_{41}	0.0008168	0.002539	0.0008055
k_{10}	0.003216	0.002489	0.002027
k_{30}	0.01136	0.002342	0.009340
V_c[c]	3.798	7.458	6.444

[a] Source: Pitmann, et al, (1976). Reprinted, with permission, from Drug Metabolism and Disposition. (c) American Society for Pharmacology and Experimental Therapeutics (1976).

[b] Values were obtained by least-squares fitting to a four-compartment, open system, mammillary model. Rate constants are days^{-1}.

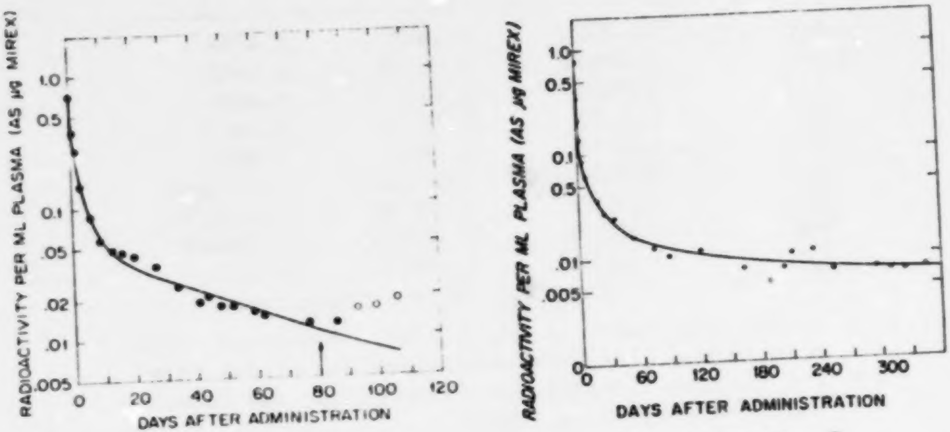
[c] V_c is in liters. V_c is the volume of distribution of the central compartment, a constant necessary to convert the plasma concentrations of mirex to quantities of mirex in the central compartment.



k_{ii}'s correspond to parameters in Table 5.15.

FIGURE 5.5. Scheme for four compartment open-system mammilary models

Source: Pittman, et al. (1976). Reprinted, with permission, from Drug Metabolism and Disposition. (c) American Society for Pharmacology and Experimental Therapeutics (1976).



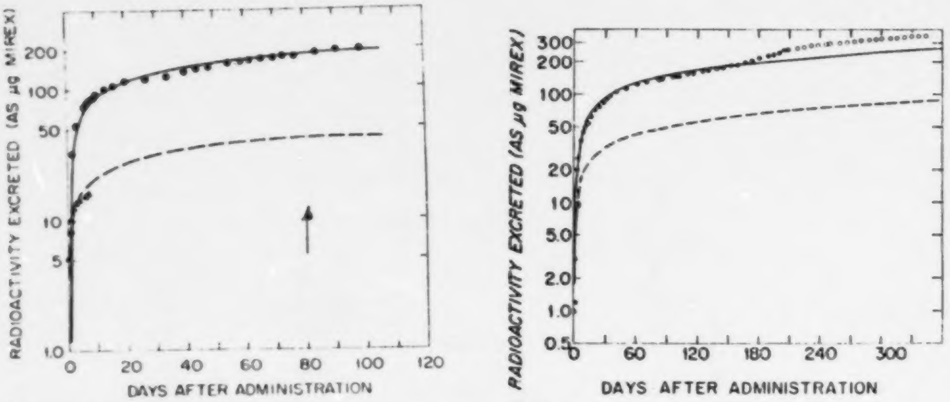
(a) Monkey No. 1

(b) Monkey No. 2

o, Experimental values used in computer fitting; o, experimental values not used in computer fitting. The line is drawn from values derived from computer fit of the four-compartment model.

FIGURE 5.6. Plasma levels of mirex vs time in monkeys after iv injection of ^{14}C-mirex.

Source: Pittman, et al. (1976). Reprinted, with permission, from Drug Metabolism and Disposition. (c) American Society for Pharmacology and Experimental Therapeutics (1976).



(a) Monkey NO. 1

Δ, Experimental urinary excretion data; o, experimental fecal excretion data. The lines are drawn from values derived from computer fit of the four-compartment model for urinary excretion (- - -), and for fecal excretion (——).

(b) Monkey NO. 2

b Δ, Experimental urinary excretion data; o. experimental fecal excretion data used in computer fitting. o, experimental fecal excretion data not used in computer fitting. The lines are drawn for values derived from computer fit of the four-compartment model for urinary excretion (- - -), and for fecal excretion (——).

FIGURE 5.7. Cumulative fecal and urinary excretion of mirex vs time in monkeys after iv injection of ^{14}C-mirex.

Source: Pittman, et al. (1976). Reprinted, with permission, from Drug Metabolism and Disposition. (c) American Society for Pharmacology and Experimental Therapeutics (1976).

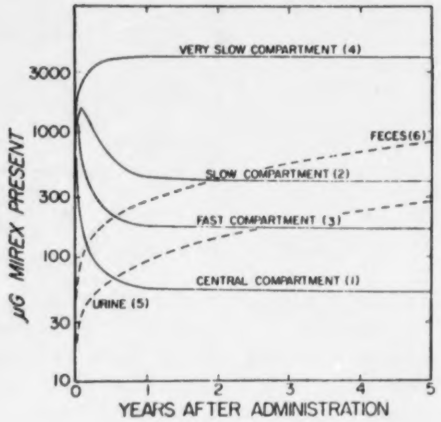


FIGURE 5.8. Calculated amount of mirex in various compartments vs time in monkey No. 2 after a single iv injection of ^{14}C mirex.

Source: Pittman, et al. (1976). Reprinted, with permission, from Drug Metabolism and Disposition. (c) American Society for Pharmacology and Experimental Therapeutics (1976).

There is very little doubt that the "very slow" compartment postulated in the model corresponds directly to adipose tissue and other especially lipid-rich and poorly perfused tissues. The "slow" compartment may correspond to moderately perfused bulk tissue such as muscle. The identity of the "fast" compartment from which mirex is excreted into the feces is presently unclear; however, the large intestine is a likely candidate in view of the high concentration of ^{14}C-mirex in this tissue. The volume of mirex in the "central compartment" probably includes the plasma and body water and perhaps other tissues which might accumulate mirex preferentially to the plasma.

Very recently, Smrek et al., (1977) reported a study of the pharmacokinetics of mirex in goats. Their experiment involved oral administration of mirex to male and female goats at various dosages and exposure regimens. Included in the study were several female goats who were observed during pregnancy and lacation. Plasma, milk and adipose tissues of the animals were analyzed for mirex at intervals.

During the course of the study (61 weeks) no obvious signs of toxicity were noted at the mirex dosage of 1 mg/kg body weight/day. The authors found that the various biological compartments (plasma, millk, adipose tissue) seem to be independent of one another. While plasma levels stabilize, adipose tissue levels increase and colostrum levels were higher than milk levels. Milk levels fluctuated, but no particular trend was discerned. The plasma levels, having stabilized, did increase in response to higher dosages, so that apparently the capacity of the plasma to transport mirex is not a limiting factor in the plasma level at dosages of 1-10 mg/kg body wt/day. One potentially important finding is that since plasma levels do not appear to increase in proportion to the concentration in adipose tissue, the determination of plasma levels in cases of chronic exposure is of little value for predicting adipose tissue levels. Furthermore, neither pregnancy nor lactation while maintaining dosage caused any decline in adipose tissue levels. There were some sex-specific differences seen: adipose tissue levels of mirex were lower in females than males. Mirex concentations in adipose tissues of males also showed greater fluctuation. Steady mirex concentrations were not reached in the course of the study (approximately 14 months) whereas the plasma levels became stationary after about 5 months.

5.3.4.2.4 Enzymatic and cellular effects--Induction of hepatic microsomal enzymes is a general characteristic of chlorinated hydrocarbon insecticides, including mirex and Kepone. Baker, et al., (1972) demonstrated this effect in both rats and mice exposed to various concentrations of mirex via intraperitoneal injection or through diet. In the injection experiments, mirex was administered intraperitoneally at dose levels of 5, 10, 25, and 100 mg/kg 72, 48, and 24 hours before the animals were killed. In the feeding experiments, mirex was added to the pelleted feed in concentrations from 1 to 250 ppm for 14 days.

Mirex treatment of both mice and rats elicited a significant increase in hepatic cytochrome P-450 levels and relative liver weight. The mice and rats treated with 25 mg/kg exhibited cytochrome P-450 levels of 2.67 and 1.55 times that of the control, respectively. Although the liver-to-body weight ratio for rats was not affected at this dosage level, mice treated at 25 mg/kg had liver-to-body weight ratios 1.26 times that of the control. A dosage of 100 mg/kg in rats produced a significant change in relative liver weight and also an increase in cytochrome P-450 levels to 2.5 times that of the controls. After 14 days, dietary dosages of mirex over 1 ppm caused induction of cytochrome P-450. At the higher dosage levels, the induction effect was much more pronounced. The cytochrome P-450 level was approximately twice the control levels at the mirex dosages of 1 to 25 ppm and 6 times the control levels at dosages of 100 to 250 ppm. In the rats tested, dietary mirex dosages of 1 to 250 ppm for 14 days resulted in increases in smooth endoplasmic reticulum. This effect was increasingly apparent as the dose level increased. The physiologic significance of this induction has not been adequately evaluated, however, interactions between induction and various drug or steroid metabolisms have been suggested.

In a study by Abston and Yarbrough (1976), adult male and female rats fed dietary mirex concentrations of 10, 20, 30, 40, and 50 ppm for 4 weeks exhibited significant decreases in liver levels of lactic acid dehydrogenase (LDH), malic dehydrogenase (MDH), sorbitol dehydrogenase (SDH), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) as compared to controls. These enzymes are associated with cellular metabolic activity. LDH is present in most animal tissues and is involved in interconversion of pyruvic acid to lactic acid, serving as a pivotal enzyme between the glycolytic pathway and the tricarboxylic acid cycle. MDH is a tricarboxylic acid enzyme involved in the conversion of malic acid to oxaloacetic acid. GOT and GPT are transaminases involved in the interconversion of metabolic intermediates relative to energy metabolism and gluconeogenesis. SDH is instrumental in conversion of glucose to fructose; elevated serum SDH levels are considered to be an indicator of liver cell damage. The observed losses of LDH, GPT, GOT and MDH were from the cytoplasmic portion of the hepatocytes rather than from the mitochrondrial portions. At equivalent mirex dosages, decreases in enzyme levels were consistently less in female rats as compared with males. The ratio of liver weight to body weight generally increased during the course of the experiment although the increases varied according to dosage level, sex, and elapsed time. The authors suggest that the increases in liver weights following mirex (and other organochlorine insecticide) exposure may represent an adaptive hypertrophy and not a toxic expression of the insecticide itself. Typically, serum enzyme levels indicate compensation reactions taking place at the organ level, from cell damage to death. The decreases in enzyme levels were both dose and time dependent. The authors believe that in an environment in which organochlorine insecticide exposure occurs continually at a low dose, these adaptive responses may become contributory factors in a population's ability to successfully meet environmental stress.

Because the LD$_{50}$ of mirex and Kepone in many species is very high, it has been commonly believed that accumulation levels represented no hazard to species which might be naturally exposed, since these accumulated concentrations were well below typical lethal doses. However Hendrickson and Bowden (1975a) have shown that LDH (crystalline from rabbit muscle) is competitively inhibited by mirex and Kepone at levels as low as 10 ppm during in vitro determinations. Both mirex and Kepone were competitive inhibitors to pyruvate; the apparent inhibition constant (K_i) was 0.02 mM for both pesticides. Figure 5.9 shows Dixon plots of the effect of mirex and Kepone on LDH activity.

The Dixon plot is a commonly used graphical method for determination of the apparent inhibition constant (K_i) in the presence of an inhibitor (Dixon and Webb, 1964). Basically, the method involves plotting the velocity of the reaction as determined for a series of inhibitor concentrations while keeping the substrate concentration constant. Hendrickson and Bowden (1975a) assayed LDH activity by following the rate of conversion of pyruvate to lactate in the presence of NADH by the loss of absorbance of NADH at 340 nm in a Beckman DB spectrophotometer.

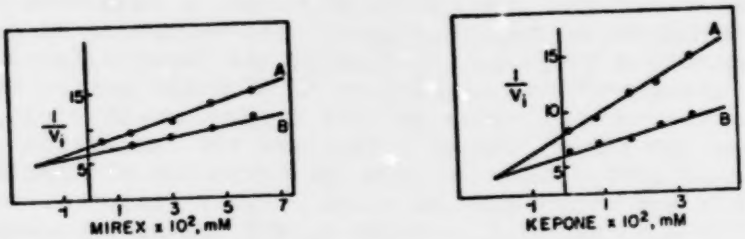


Figure 5.9 Dixon plots of effect of mirex and Kepone on LDH activity.

Source: Hendrickson and Bowden (1975). Reprinted, with permission from J. Agricultural and Food Chemistry.

As shown in Figure 5.9, the concentration of pesticide, in millimoles, is plotted against $1/V_i$ where V_i is the initial velocity in millimoles of NADH (the reduced form of coenzyme I) consumed per minute. Varying concentrataions of pesticide were added to cuvettes containing pyruvate (A = 0.66 mmol, B = 1.3 mmol for mirex, and A = 0.33 mmol, B = 0.66 for Kepone), 0.7 mmol of NADH, 10 percent ethanol, and 0.1 M phosphate buffer (pH 7.5) in a volume of 3.0 ml.

A straight line was obtained by plotting pesticide concentration vs $1/V_i$. Lines A and B represent the effects of various concentrations of mirex or Kepone added to cuvettes containing pyruvate at either of two concentrations (A or B), while keeping the concentration of NADH, ethanol and phosphate buffer constant. The intersection of Lines A and B (each representing a series of inhibitor concentrations having a constant substrate concentration) gives the inhibition constant.

The observed inhibition occurred at test levels well below usual lethal doses and was comparable, in fact, to accumulation concentrations. LDH is common to many species which participate in man's food chain. Because LDH occupies a key position in the anaerobic glycolytic pathway of skeletal muscle, impairment of the function of this enzyme in energy production could result in possible harmful effect on muscle function.

Using LDH as a model system, Hendrickson and Bowden (1975b) proposed a mechanism for the *in vitro* inhibition of NADH linked dehydrogenase by halogenated hydrocarbon pesticides, including mirex. This mechanism includes (1) the association of NADH and mirex, and (2) binding of mirex with the adenosine-binding pocket of LDH (because of the hydrophobic character and structural similarity of mirex in comparison with other competitive inhibitors of dehydrogenases). The mechanism is consistent with the inhibition patterns obtained by the investigators in their studies of time-dependent association of NADH and pesticide and the postulation that mirex binds at the adenosine-binding pocket of dehydrogenases. The theory not only includes the inhibition of LDH by polychlorinated pesticides, but also the inhibition of other NAD-linked dehydrogenases and other enzymes which utilize adenosine-containing coenzymes: FAD(H_2), NADP(H_2), coenzyme A, ATP, and ADP. A scheme for the actual *in vivo* mechanisms by which polychlorinated hydrocarbons exert their lethal neurophysiological effects is proposed. This mechanism includes the disruption of nerve membrane-bound ATPases (which maintain intracellular/extracellular ion ratios) by pesticide binding at the adenosine-binding pocket of the ATPase.

A common responses of liver cells to toxic stress is the accumulation of lipids, usually in the form of triglycerides. Kendall (1974a) reported a study in which a lipophilic stain, Sudan black, was utilized to visualize fat inclusions in the livers of rats. The single LD_{50} mirex dosage of 365 mg/kg, when administered intraperitoneally, produced several distinctive histologic alterations in the liver after 12 days. These changes included apparent depletion of stainable glycogen, hepatocyte enlargement, periportal liposis associated with central necrosis and infrequent foci of necrosis on the surface. When the LD_{50} dosage was fed *ad libitum* all of the above types of injuries except surface lesions occurred. Mirex caused lipid accumulation in an extremely distinctive histologic pattern of periportal liposis. No sudanophilic positive material was observed in liver tissue of unexposed controls. Kendall (1974b) is currently investigating the cirrhogenic potential of mirex in light of these findings.

Mirex has been shown to increase liver weight and to produce a proliferation of smooth endoplasmic reticulum and an incease in hepatic mixed function oxidases in the rat Baker, et al., (1972) Abston and Yarbrough, (1976), Gaines and Kimbrough, (1970) . An article by Byard, et al., (1975) extended these findings to mice and suggested that a large component of the liver growth produced by mirex in the mouse is due to a stimulation of DNA synthesis which leads to overall cellular growth. An early, controlled response of DNA to the growth stimulus resulted in overall cellular growth of about 130 to 150 percent of control values. Beyond this level, and as a result of further cellular changes produced by mirex or aging, a more rapid increase in DNA was initiated which was associated with the appearance of nodules. Byard, et al., (1975) offer an interesting hypothesis to explain the action of lipophilic chemicals (such as mirex and Kepone) in proliferating the smooth endoplasmic reticulum. According to the hypothesis, these chemicals mimic a lipophilic substance in the body which normally controls the synthesis of components of the smooth endoplasmic reticulum. For example, steroid hormones have been shown to bind to specific receptor proteins in the nucleus, and stimulate RNA synthesis. Similar mechanisms have been proposed for chemical inducers of smooth endoplasmic reticulum proliferation. The fact that mirex binds to soluble cytoplasmic proteins in the liver is consistent with this hypothesis.

5.3.4.2.5 Reproductive effects--Laboratory studies have repeatedly demonstrated that mirex and Kepone are capable of producing similar adverse reproductive effects in numerous species. Both pesticides have been associated with the following effects:

(1) Reductions in fertility and litter size
(2) Increased rate of fetal visceral anomalies
(3) Elevated pesticide levels in tissue of fetuses and offspring as a result of placental transfer and/or lactogenic exposure
(4) Decreases in birth weight and survival rate of young.

In addition, lactogenic exposure to mirex has been associated with the development of cataracts in the young.

Gaines and Kimbrough (1970) fed mirex at dosages of 5 and 25 ppm to Sherman rats for 45 and 102 days. Reproductive activity of male rats (fed 25 ppm) and females (fed 5 ppm) bred to untreated rats was comparable with that of controls. Females fed 25 ppm mirex for 45 days and then bred to non treated males had litters significantly smaller than those of controls. The survival rate to weaning was significantly reduced and 33 percent of the offspring developed cataracts. Compared with controls, females bred after 102 days' exposure to mirex had fewer litters, the survival rate of the offspring was significantly lower (P 0.05) and 46.2 percent of the offspring developed cataracts. No cataracts were observed in offspring of control rats nor in the parent stock fed mirex.

Gibson, et al., (1972) also reported that for Sherman rats fed diets containing 25 ppm of mirex for as little as 45 days fewer offspring were born alive, fewer offspring survived to weaning, and many pups developed cataracts. Analyses of milk and fetuses showed excretion of mirex in the milk and passage through the placental barrier. Another study by Ware and Good (1967) investigated the reproductive effects of mirex on two strains of mice. In all instances, mirex treatment resulted in a reduction of litter size and number of offspring produced per day. The effect failed to achieve statistical significance. In one strain of mice, mirex produced a significant increase in parent mortality; however, in the other strain this effect was not observed.

5.3.4.2.6 Placental and lactogenic transfer--In a study of placental transfer of mirex in rats, Gaines and Kimbrough (1970) examined fetuses taken from rats treated for 78 days. The fetuses contained 0.14-0.45 ppm of mirex; no mirex was detected in fetuses from controls. Khera, et al., (1976) have also documented placental transfer of mirex. Gaines and Kimbrough (1970) also provided evidence of excretion of mirex in milk and the toxic effects of lactogenic exposure to suckling rats. The survival rate to weaning of rats, transferred at birth from dams fed non-treated diet to foster mothers also fed the non treated diet, was 95.8 percent; none developed cataracts. The survival rate of rats exposed to mirex *in utero* only did not differ significantly from that of controls and only 1.6 percent developed cataracts. When rats born to non-treated mothers were exposed postpartum to mirex-treated foster mothers until weaned, their survival rate was only 53.9 percent or 41.9 percent less than that for controls; 37.5 percent developed cataracts. The low survival rate of offspring of treated dams was caused chiefly by the high mirex content in the mothers' milk. The mean mirex content of milk taken from the stomachs of suckling rats was 11.3 ppm.

5.3.4.2.7 Teratogenic effects and dominant lethal study--A recent investigation by Khera, et al., (1976) has demonstrated the teratogenic potential of mirex in rats. Low doses of mirex (1.5 or 3.0 mg/kg given as a single daily oral dose on days 6 to 15 of gestation) produced neither adverse fetal effects nor symptoms of maternal toxicity. However, dosages of 6.0 or 12.5 mg/kg administered according to the same protocol caused maternal toxicity and increased fetal visceral anomalies. At the 12.5 mg/kg dose level, decreased fetal survival and reduced fetal weight also occurred.

A dominant lethal study also reported by Khera, et al., (1976) did not demonstrate significant differences in reproductive parameters between male control rats and experimental groups of male rats fed 0, 1.5, 3.0 or 6.0 mg/kg daily for 10 days before mating. Mirex was detected in the testes of the experimental group, however.

5.3.4.2.8 Carcinogenic and tumorigenic effects--Innes, et al., (1969) examined mirex as part of a large scale study designed to screen selected pesticides and industrial compounds for tumorigenicity. Mirex was tested by oral and subcutaneous routes of administration in two hybrid strains of mice. Eighteen male and 18 female mice of each strain were utilized for each test procedure. In the subcutaneous test, mirex was administered in a maximally tolerated single dose of 1000 mg/kg. In the oral experiments, a dose of 10 mg/kg/day was given from the seventh to the twenty-eighth day. The mice were then fed mirex at the level of 26 ppm in the diet for the duration of the experiments. All mice in both experiments were sacrificed approximately 18 months after the beginning of the experiment. The search for effects included an external examination and a thorough examination of thoracic and abdominal cavities, with histologic examination of major organ and all visible lesions. Evaluation of tumorigenicity was based on magnitude of the relative risk, comparing tumor incidence among the treated mice with that of the controls. A separate analysis was performed for each of four tumor groupings: hepatomas, pulmonary tumors, lymphomas and total mice with tumors. Findings are summarized in Tables 5.16 and 5.17. Hepatic tumors were the only type observed in the mirex-fed mice.

In the oral administration tests, all mice died prior to the completion of the experiment at 18 months. Twenty-nine of 72 mice developed hepatomas, compared to 14 of 338 control mice, a difference significant at P 0.01. The authors stated that mirex induced a highly significant (P 0.01) increase in liver hepatomas in both strains of mice tested. Liver tumors were diagnosed as carcinomas. The classification "hepatomas" in Tables 5.16 and 5.17 also includes metastasizing hepatic cell tumors and should not be taken to imply that the tumors were benign.

The significance of this bioassay is strengthened by the fact that seven known carcinogens, included in the study as positive controls, were found clearly tumorigenic by this test protocol. Mirex by oral administration was judged to have a relative risk of 0.945 by comparison with an average of seven known carcinogens, meaning that in this bioassay procedure mirex possesses approximately 95 percent of the carcinogenic potency of the seven known carcinogens tested.

A second carcinogenesis bioassay of mirex was conducted using Charles River Caesarian derived (CD) rats. Mirex was administered in the feed to groups of 26 rats of each sex at levels of 50 and 100 ppm for 18 months (Ulland et al., 1973; Ulland et al., 1977). Untreated controls as well as a group of positive controls treated with a known carcinogen (2-AAF) were included for comparative purposes.

At the time of the initial evaluation (Ulland et al., 1973) it was reported that mirex was not carcinogenic in rats, however subsequent re-analysis of the data (Ulland et al., 1977) using newer guidelines on the classification of liver tumors indicated that mirex appears to be carcinogenic. The controversy surrounding the pathologic classification of liver neoplasms was discussed in the December 29, 1976 issue of the Federal Register.

The test animals showed a wide spectrum of neoplasms which, except for liver lesions, showed little correlation with mirex dosage. Therefore, the relationship between mirex exposure and the development of these lesions could not be established.

TABLE 5.16. RELATIVE RISK FOR DEVELOPMENT OF TUMORS AMONG MICE TREATED WITH MIREX WHEN COMPARED WITH CONTROLS[a]

	Strain[b]	Mice with hepatomas			Mice with pulmonary tumors			Mice with lymphomas			Total mice with tumors		
		M	F	Sum	M	F	Sum	M	F	Sum	M	F	Sum
	X	4.37[c]	Inf.[d,e]	7.33[d]	0	0	0	0	0	0	1.67	9.35[d]	3.53
Mirex	Y	8.14[d]	87.78[d]	18.12[d]	0	0	0	0	0	0	2.37	16.26[d]	5.85
	Sum	5.74[d]	107.39[d]	11.54[d]	0	0	0	0	0	00	1.95	12.20[d]	4.46

[a] Adapted from Innes, et al., (1969).

[b] Strain X-(C57BL/0 x C3II/Anl)F_1: strain Y-(C57BL/6 x AKR)F_1.

[c] Increased tumor yield significant at 0.05 level.

[d] Increased tumor yield significant at 0.01 level.

[e] Inf.--relative risk calculated as infinite. This figure may result from the absence of tumors in the control group and is not necessarily significant.

TABLE 5.17. TUMORS AMONG MICE RECEIVING MIREX[a]

Strain	Total mice necropsied		Weeks at necropsy		Mice with hepatomas		Mice with pulmonary tumors		Mice with lymphomas		Total mice with tumors	
	M	F	M	F	M	F	M	F	M	F	M	F
X	18	16	59	70	6	8	0	0	0	0	7	8
Y	15	16	59	69	5	10	0	0	0	0	5	10

[a] Adapted from Innes, et al., (1969).

There was a high incidence of tumors, regardless of sex or dose, but no statistically significant differences were noted except for tumors of the liver (neoplastic nodules). One carcinoma was detected in the low dose group, five in the high dose group and none in the control group. The differences in this parameter between test animals and controls were not significantly different from controls. Only the observation of 7 neoplastic nodules in the high dose (100 ppm) male rats was significant at the 0.05 level. The authors did state, however, that this type of neoplastic nodules has been shown to progress to hepatocellular carcinoma in studies of other model carcinogens. In addition, the absence of neoplastic nodules among animals unexposed to mirex and the fact that this type of lesion is characteristic of early response to known carcinogens is also suggestive of carcinogenic activity. According to the authors, to call such nodules merely hyperplastic belies their neoplastic nature and malignant potential.

5.3.4.3 Kepone--

5.3.4.3.1 Acute toxicity--In experimental animals (rats), the acute oral and dermal LD_{50} of Kepone was 95 to 140 and 250 mg/kg, respectively. In male rabbits, the acute dermal LD_{50} was 345 to 475 mg/kg. DDT-like tremors developed in these animals following a single dose, reaching a peak intensity about the second day and persisting for about 1 week or more (U.S. Environmental Protection Agency, 1975).

The acute oral LD_{50} of powdered technical Kepone (94.0 percent) when administered as a 5 percent solution in corn oil to several species is shown in Table 5.18. The characteristic effect of this compound was the development of DDT-like tremors.

TABLE 5.18. ACUTE ORAL TOXICITY OF KEPONE TO VARIOUS MAMMALS[a]

Animal	Kepone dosage LD_{50}
Male rats	132.0 ± 8 mg/kg
Female rats	126.0 ±12 mg/kg
Male rabbits	71.0 ± 6 mg/kg
Dogs	250.0 mg/kg

[a] Source: U.S. EPA (1975).

Acute rat inhibition studies of 10 percent Kepone dust at levels of 2 and 10 times those that are likely to occur under agricultural conditions produced no observable adverse effects. Concentrations chosen were based upon observations made in actual use tests of the dust, indicating a concentration of 1.6 mg Kepone active ingredient per cubic meter of air inhaled. Two experimental groups were exposed to 10 percent Kepone dust at levels of 3.7 mg/m^3 of air and 15.4 mg/m^3 . Ten daily exposures of about 2 hours were conducted each, for a total of about 19 hours (U.S. Environmental Protection Agency, 1975).

The acute dermal LD_{50} in male rabbits of the powdered technical Kepone administered as a 20 percent solution in corn oil was 410 ± 65 mg/kg. Severe and persistent tremors developed with little or no skin irritation. The LD_{50} of the undiluted formulation was 435 ± 11 mg/kg. The formulation caused marked irritation and edema, followed by the development of a hard scab which eventually sloughed off and revealed new and apparently healthy skin.

5.3.4.3.2 Subacute and chronic toxicity--Huber (1965) investigated the physiological effects of Kepone in the laboratory mouse. Subacute mortality tests (tolerance tests) were conducted by feeding 0, 10, 30, 40, 60, 70, 80, and 100 ppm Kepone to juvenile and adult mice of both sexes. Results showed that 80 ppm or higher was lethal to all adults tested within 32 days, whereas 70 ppm was lethal to weaned juveniles within 19 days. No mortality occurred in adults or juveniles fed 40 ppm in tests extending up to 12 months. Food and water consumption of mice fed 40 ppm or more of Kepone increased 20 to 40 percent with no increase in body weight. Subacute lethal levels adversely affected the growth of juveniles and caused weight loss in adults. Results are summarized in Table 5.19. Constant tremor syndrome appeared within 4 weeks in all mice fed 30 ppm or more; however, this terminated within 4 weeks following withdrawal from the treated diet. Accumulation patterns are shown in Table 5.20. The liver was the only organ which showed an increase in size in all animals fed 30 ppm or more. At 40 ppm, the liver weight doubled in 60 to 90 days; it decreased in size when the treated diet was withdrawn. Histologic findings at necropsy were focal necrosis, cellular hypertrophy, hyperplasia and congestion, the degree of which depended on length of treatment.

Kepone residues in the organs of mice showed maximum accumulation within 5 months with no additional increase at a dietary level of 40 ppm Kepone (Table 5.21). The liver was the major organ of accumulation while the gonads, adrenals, uterus, spleen and heart showed little increase in Kepone accumulation after the first 30 days of treatment. Further Kepone accumulation took place mainly in the liver, brain, kidneys and body fat. After withdrawal from the treated diet, Kepone residues decreased rapidly from the various organs. As much as 56 percent of the accumulated Kepone was lost by 24 days and up to 95 percent by 150 days (Table 5.22).

Effect of Kepone on male rats was demonstrated in a study in which rats were given 9.6 mg/kg per rat per day for 20 days. Neurological

TABLE 5.19. SUMMARY OF KEPONE MORTALITY STUDIES WITH THE LABORATORY MOUSE[a]

Kepone in Diet, ppm	Age	Number	Initial death, days	Cumulative mortality at indicated days of treatment, percent			
				25	50	75	100
50	Adults	8	70				
	Juveniles	4					
60	Adults	8	107				
	Juveniles	4	75	75	87	92	98
70	Adults	16	17	19	21	42	
	Juveniles	8	5	5	9	12	15
80	Adults	8	5	10	14	15	25
	Juveniles	8	5	9	11	13	17
100	Adults	70	6	12	16	20	32
	Juveniles	8	2	5	10	13	19

[a] Source: Huber (1965). Reprinted, with permission, from Toxicology and Applied Pharmacology. (c) Academic Press, Inc. (1965).

TABLE 5.20. LIVER WEIGHT AS A PERCENTAGE OF BODY WEIGHT IN KEPONE-FED AND CONTROL MICE[a]

Kepone in Diet, ppm	Days on Kepone diet	Days on control diet	Number of mice	Percent body weight
0			60	6.0
40	7- 14	0	10	7.8
40	21- 42	0	8	9.2
40	56- 60	0	3	12.0
40	90	0	5	11.8
40	120-150	0	6	12.6
40	180-210	0	21	13.5
40	240-270	0	28	13.3
40	500	0	2	15.0
100	7	0	8	10.2
100	14	0	9	12.4
100	21- 28	0	15	13.5
40	210	120	9	8.8
40	60	120	8	7.1

[a] Source: Huber (1965). Reprinted, with permission, from Toxicology and Applied Pharmacology. (c) Academic Press, Inc. (1965).

TABLE 5.21. RESIDUES OF KEPONE IN MOUSE ORGANS AT A DIETARY LEVEL OF 40 PPM[a]

Days on 40 ppm Kepone	Kepone, ppm						
	Liver	Brain	Kidney	Fat	Muscle	Adrenals	Gonads
5	45	3	7	13			
15	39	20	30	27	5		
30	78	26	27	25	20	67	25
30	64	30	32	33	15	25	25
90	67	29	27	29	15	15	13
90	66	30	39	34	16	37	
150	168	26	74	81	23	45	26
150	90	61	37	49	19	86	
500	120	75	88	84	26	72	20
500	96	55	35	29	11	20	18
100[b]	60	25	25	41	10	25	26
300[b]	113	65	49	22	8		17

[a] Source: Huber (1965). Reprinted, with permission, from Toxicology and Applied Pharmacology. (c) Academic Press, Inc. (1965).

[b] Males, all others are females.

TABLE 5.22. RESIDUES OF KEPONE IN MOUSE ORGANS AFTER WITHDRAWAL OF 40 PPM DIET[a]

Sex[b]	Days on Kepone diet	Days on control diet	Kepone, μg			
			Liver	Brain	Kidneys	Total
F	5	0	67.5	1.2	1.8	70.5
F	15	0	77.0	7.2	8.4	92.6
F	30	0	140.0	13.5	6.7	160.2
F	30	0	96.0	10.7	7.7	114.4
F	90	0	160.0	11.5	6.0	177.5
F	90	0	138.7	11.0	8.5	158.2
F	150	0	520.0	8.3	22.8	551.1
F	150	0	335.0	25.5	13.4	373.9
F	500	0	418.0	30.0	29.0	477.0
F	500	0	403.2	23.5	9.0	435.7
M	100	0	189.0	10.5	11.7	211.2
M	300	0	450.0	26.0	24.0	500.0
F	90	14	125.0	5.4	1.0	131.4
F	90	24	59.4	5.4	6.5	71.3
F	60	150	1.4	0	0	1.4
F	210	150	0.8	0	0	0.8
M	210	150	9.4	0	0	9.4
M	150	240	1.2	0	0	1.2

[a] Source: Huber (1965). Reprinted, with permission, from Toxicology and Applied Pharmacology. (c) Academic Press, Inc. (1965).

[b] M = male; F = female.

effects (see Section 5.3.4.3.4) were also characteristics of Kepone-dosed rats. Tremors appeared in all treated animals after the fifth dose. Survivors of the 20-day study showed gradual subsiding of the tremors. No lesions were found on histopathologic examination of selected organs (U.S. Environmental Protection Agency, 1975). In a related long-term study, six groups of rats (mixed sexes) were fed 5, 10, 25, 50, 80 ppm of Kepone for 3 months. Food consumption, hematologic and urinary data were collected. Organ weights and samples of organs and fat were also collected for analyses of Kepone.

Tremors occurred in females but not in males after 3 months at the dietary level of 25 ppm Kepone. Rats receiving 50 and 80 ppm showed evidence of tremors as early as 2 weeks. Sugar in urine was negative and trace quantities of protein were present. At 80 ppm, blood platelet counts, serum calcium, prothrombin clotting time and (Factor V) accelerator globulin were not significantly different from those of controls. Survival was adversely affected at the 80 ppm level. Weight depression became significant at dosages between 5 and 10 ppm in females and between 10 and 25 ppm in males. Organ-to-body weight ratios were significantly higher for liver in females at 5 ppm and males in the range of 5 to 10 ppm. Kidney-to-body weight ratios were significantly elevated in females at 10 ppm or greater and in males at 50 ppm. Similar ratios for testes became significantly low at 50 ppm. Storage of Kepone in fat generally increased with increased feeding levels of Kepone (U.S. Environmental Protection Agency, 1975).

Details of the 12-month chronic rat study were the same as the previous (3-month) study. After 7 months, female rats fed 10 ppm were highly excitable but no muscle tremors were observed. Urine analyses for sugar at 6, 9, and 12 months were negative at feeding levels of 5, 10, and 25 ppm. Increased protein excretion was noted with increased dietary levels of Kepone. Female growth was progressively reduced as dietary Kepone increased above 5 to 10 ppm. Among males, there was no appreciable effect on growth or survival at 5 to 10 ppm, but both sexes were affected at 25 ppm. Oxygen consumption of both sexes at 25 ppm differed significantly from controls, possibly resulting from an elevated metabolic rate. Trends for organ to body weight ratios were similar to those observed at 3 months. Lowered hematocrit and increased intake were also noted.

Animals sacrificed at 12 months revealed liver enlargement due to congestion, probably attributable to increased cardiac output and raised metabolic rate which often accompany tremors. Necrosis was absent in the nervous system nor were any unusual kidney lesions seen which could account for the observed elevations in urinary protein.

In rats surviving between 1 and 2 years, two tissues, liver and kidney, exhibited lesions that were subjected to special study and interpretation. In the liver, the question concerned the possible carcinomatous nature of lesions seen in six rats (three females on 10 ppm, one female on 25 ppm and two males on 25 ppm). Following independent review by four pathologists, this possibility remains an equivocal one. The National Cancer Institute (NCI) is presently testing Kepone for carcinogenicity and a final report may be forthcoming in the near future. Results of the preliminary report from NCI are discussed in Section 5.3.4.3.8.

A study in which groups of purebred beagle dogs were fed Kepone in their diets for periods up to 127 weeks has been reported by the U.S. Environmental Protection Agency (1975). Kepone levels in food were 0, 1, 5, and 25 ppm. During the first year, body weight gains were similar among all groups, however, subsequent weight gains were significantly lower in the dogs on the 25 ppm dosage. Food consumption data indicated decreased efficiency of food utilization in the treated dogs as compared with that of controls, possibly resulting from an increased metabolic rate, an effect which has been demonstrated in rats. No effects on hematocrit, hemoglobin, white cell counts, urinary sugar or protein, or liver function were observed.

Statistically significant differences in organ-to-body weight ratios were apparent for three organs at the 25 ppm dosage: the liver, the kidneys and the heart. The increases in heart and kidney ratios may be explained by the poor nutritional status of these animals (there was a virtual absence of fat) rather than any specific physiologic response to Kepone. The liver differences, on the other hand, were thought to reflect a toxic effect specifically related to Kepone (rather than underweight).

Kepone fed to dairy cattle for 60 days at dietary levels of 0.25, 0.5, 1.0, and 5.0 ppm produced no apparent ill effects (Smith and Arant, 1967). Cows fed freely, gained weight, and produced a normal amont of milk, and three cows dropped normal calves. Kepone was present in measurable quantities in milk 1 week after the cows were placed on the treated rations. There was a gradual increase in the amount of Kepone in milk during the first few weeks and a leveling off in Kepone content at the end of approximately 2 months. The highest residue reported in milk was 0.44 ppm. Thirty-five days after removal from the treated rations, cows formerly on the lowest ration (0.25 ppm) excreted less than 10 ppb Kepone in their milk; similarly, those on the highest rate excreted approximately 90 ppb. Eighty-three days following the cessation of Kepone feeding, no measurable amount of Kepone was present in the milk of cows fed the lowest rate and only traces were found in milk from cows fed the highest rate.

5.3.4.3.3 Metabolism--Huber's (1965) residue studies, described in the previous section, failed to reveal any Kepone metabolite in the organs of mice fed mirex. This work provided the basis of the hypothesis, since confirmed, that Kepone is very stable, a property which may enhance rapid accumulation, especially in the liver and brain (Huber, 1965).

Recent radioactive tracer studies of Kelevan (ethyl Kepone-5-levulinate) metabolism, showed that one metabolic product is Kepone (U.S. Environmental Protection Agency, 1976a). Thirteen weeks after administration, the distribution of Kepone in rat tissues was as follows: Liver kidney fat muscle. Information on blood concentrations was not available. The reported data indicated that the biological half-life of Kepone in rats is approximately 4 weeks. In addition, the possible fat-plasma partition coefficient for Kepone is low in comparison with other chlorinated hydrocarbon insecticides, making more of the compound available to the liver for metabolism and to the kidney for excretion. Since a high concentration of a component calculated as ^{14}C-labeled derived Kelevan was found in the colon contents, intrahepatic recirculation of this compound and Kepone and/or elimination is suggested.

5.3.4.3.4 Neurologic effects--The most characteristic neurological effect of Kepone appears to be the induction of DDT-like tremors. The severity of this effect (as with DDT and other chlorinated insecticides) is dosage and time dependent. This response is common to most species tested, including chickens, ducks, rabbits, mice and rats (and in man, as evidenced by medical records of Life Science Products Company employees, discussed in Section 6.4.2).

At sufficiently high dietary levels of Kepone, tremors have been evoked in experimental animals, as shown below: (U.S. Environmental Protection Agency, 1975).

Animal	Dosage	Time to Tremor	Effect
Rat	9.6 mg/kg	5 doses	Severe tremors
Rats	80 ppm	2 weeks	Tremors
	50 ppm	3 weeks	Tremors
Rats (female)	25 ppm	3 months	Tremors upon stimulation
Mice and rats	26-40 ppm	--	Tremors accompanied by dermatologic changes

In some of these tests, effects were seen to be reversible upon cessation of feeding.

A report from the U.S. Environmental Protection Agency (1976a) discusses the physiological mode of action of DDT and other chlorinated hydrocarbon insecticides. Neurophysiological experiments have demonstrated that these compounds exert their effect by prolonging the flow of sodium (Na^+) ions and interfering with the flow of potassium

(K^+) ions or by producing derangement in sodium and potassium conductance during axonic conductance. Thus, the mechanics of muscle contracture is affected by changes in the action potential.

5.3.4.3.5 Reproductive effects--Chronic doses of Kepone produce effects on reproduction at levels ranging from 5 to 80 ppm. Huber (1965), in his study described earlier, observed that the major adverse physiologic effects resulting from ingestion of sublethal levels of Kepone, exclusive of the liver pathology and tremor syndrome, involved the reproductive processes. The female hormonal system was disturbed, symptoms included: constant estrus, development of large follicles, and absence of corpora lutea, probably resulting from prolonged stimulation of FSH and estrogen but insufficient LH stimulation. Numbers of viable offspring produced were reduced 23.9, 79.3, and 87.0 percent at 10, 30, and 37.5 ppm, respectively. Matings between Kepone-fed females and control males failed to produce any litters; however, control females did produce litters when mated with Kepone-fed males. After resumption of the untreated diet, reproduction resumed within 7 weeks. Initial litters were smaller and had greater mortality than controls, indicating that accumulated Kepone may exert a detrimental effect. Second litters were larger with no greater mortality than control litters (Table 5.23).

A separate and independent mouse reproduction study (Good, et al., 1965) showed that reproduction in mice was affected at all Kepone feeding levels (10 ppm to 37.5 ppm) and that there was a decrease in size and number of litters. Increased dose levels resulted in more severe effects (U.S.Environmental Protection Agency, 1976a). In addition, a 3-month rat study reported in Allied Chemical Corporation's Pesticide Tolerance Petition revealed testicular atrophy in male rats at dosage levels of 25, 50, and 80 ppm (U.S. Environmental Protection Agency, 1976b).

Gastric intubation of 12 and 24 mg/kg/day for 4 consecutive days after birth resulted in a reduction of litter viability in studies at the Health Effects Research Laboratory (HERL) at Research Triangle Park on the effect of Kepone on reproduction in mice. (U.S. Environmental Protection Agency, 1976a). In rats, 10 mg/kg/day resulted in a reduction in litter weight. In a male fertility study, adult male rats were orally administered Kepone at a daily dose of 10 mg/kg body weight for 10 days. The dose-regimen was shortened to 6 days due to the symptoms of severe neurological impairment before the fifth day. The dosed rats were then mated with virgin females. Preliminary results indicate that this dose pattern causes:

TABLE 5.23. REPRODUCTION DATA FOR 100 DAYS OF KEPONE-FED AND CONTROL MICE[a]

Kepone in Diet, ppm	Pair	Litters		Average young per litter	Survival, Percent	Pair days per litter	Pair days per young
		1st	2nd				
				Group A[b]			
0	8	7	5	7.7	89	67	8.7
10	8	6	4	7.1	87	80	11.3
30	8	4	0	4.7	26	200	42.1
37.5	8	3	0	4.0	42	267	66.7
				Group B[c]			
0	14	14	14	7.1	89	50	7.0
40	14	0	0	---	--	---	---

[a] Source: Huber (1965). Reprinted, with permission, from Toxicology and Applied Pharmacology. (c) Academic Press, Inc. (1965).

[b] Fed indicated diet 1 month before mating and during test period.

[c] Fed indicated diet 2 months before mating and during test period.

(1) An immediate and significant loss of fertility expressed as a capability to impregnate and produce live young. The loss of fertility is temporary and returns by the third week after dosing, up to 55 days.

(2) Neurological symptoms (tremors, hyperexcitability) after 4 days' dosing which continues for about 14 days.

(3) Immediate weight loss with statistically significant differences evident 75 days after dosing.

5.3.4.3.6 Placental and lactogenic transfer--Placental and lactogenic transfer of Kepone has also been documented (Huber, 1965). After feeding pregnant mice 40 ppm of Kepone in the diet, Huber found an average of 5 ppm accumulated in seven embryos weighing approximately 0.3 g. This level is comparable to that in the maternal brains but is considerably lower than the levels found in maternal livers (45 ppm) and maternal fat (13 ppm). Four placenta averaged 12 ppm Kepone. The body of a 6-day-old suckling mouse contained 3 ppm Kepone after its mother had been fed 40 ppm Kepone the previous days.

5.3.4.2.7 Teratogenic effects--Chernoff and Rogers (1976) reported fetal toxicity in rats and mice exposed to Kepone in utero. Doses of 10, 6, and 2 mg/kg/day of Kepone in rats and 12, 8, 4, and 2 mg/kg/day in mice were administered to pregnant animals by gastric intubation during the major period of organogenesis--Days 7 to 16 of gestation. Fetal toxicity occurred in both rats and mice at doses which caused significant reductions in maternal weight gains during gestation and increased liver/body weight ratios. In rats, fetuses from dams receiving the highest dose (which also resulted in 19 percent maternal mortality) exhibited significantly higher incidence of low birth weight, reduced degree of ossification, edema, undesecended testes, enlarged renal pelvis, and enlarged cerebral ventricles. Lower dose levels, which produced maternal weight loss and liver/body weight ratio increase but no mortality, caused only reduced fetal weight and reduced degree of ossification in offspring. Male rats born to dams treated with Kepone during gestation showed no reproductive impairment. In the mouse, fetotoxicity occurred only in the highest dose group and was manifested by increased fetal mortality and clubfoot.

5.3.4.3.8 Carcinogenic and tumorigenic effects--The National Cancer Institute (U.W. DHEW, 1976) has issued a report confirming the carcinogenicity of technical grade Kepone in rats and mice. Two levels of Kepone were adminisstered in the diet of both rats and mice for 80 weeks. At the beginning of the experiment, dosage for all animals was higher but was reduced subsequently because the initial dosages were not well tolerated. The average dosage over the course of the experiment was as follows:

	Kepone in Diet, ppm			
	Mice		Rats	
	High	Low	High	Low
Male	24	8	23	20
Female	26	18	40	20

The rats were sacrificed and autopsied at 112 weeks and the mice at 90 weeks. Clinical signs of toxicity were observed in both species, including generalized tremors and dermatologic changes. A significant ($P < 0.05$) increase was observed in the incidence of hepatocellular carcinomas in rats at the high dose level and mice at both dose levels of Kepone. The incidences in the high dose groups were 88 percent and 47 percent for male and female mice, respectively, and 7 percent and 22 percent in male and female rate, respectively (Figure 5.10). No hepatocellular carcinomas were observed in control rats.

The control mice had 16 percent incidence among males. No hepatocellular carcinomas were observed in the female controls. Among the low dose groups of mice, the incidences were 81 percent for males and 52 percent for females, as shown in Figure 5.10. Importantly, the time to detection of the first hepatocellular carcinoma observed (at death) was shorter for treated than control mice and, in both sexes of both species, it appeared to be inversely related to dose. Extensive hyperplasia of the liver was also found among Kepone-treated animals of both species. The Kepone-treated animals did not differ significantly from controls as to tumors in sites other than the liver. This report presents a clear indication of Kepone's oncogenicity.

Data submitted by Allied Chemical Corporation also indicated that Kepone is oncogenic in rats (U.S. Environmental Protection Agency, 1976b). Twelve groups of 40 male and female albino rats of an unspecified strain were fed 0, 5, 10, 25, 50, and 80 ppm Kepone for periods of up to 2 years. Only seven male rats were examined at the 25 ppm dose level. Four clinical pathologists examined the slides made from the liver tissues of these treated rats. Liver lesions in one rat were diagnosed as hepatocellular carcinomas by two pathologists and "evolving carcinomas" by one pathologist who also found "evolving carcinomas" in a second male rat at this feeding level. Of the 16 female rats surviving at the 10 ppm feeding level, liver lesions in three were diagnosed as hepatocellular carcinomas by one pathologist. Of the nine female rats surviving at the 25 ppm level, liver lesions in one were diagnosed as "evolving carcinomas" by one pathologist. None of the 23 control rats developed hepatocellular carcinomas.

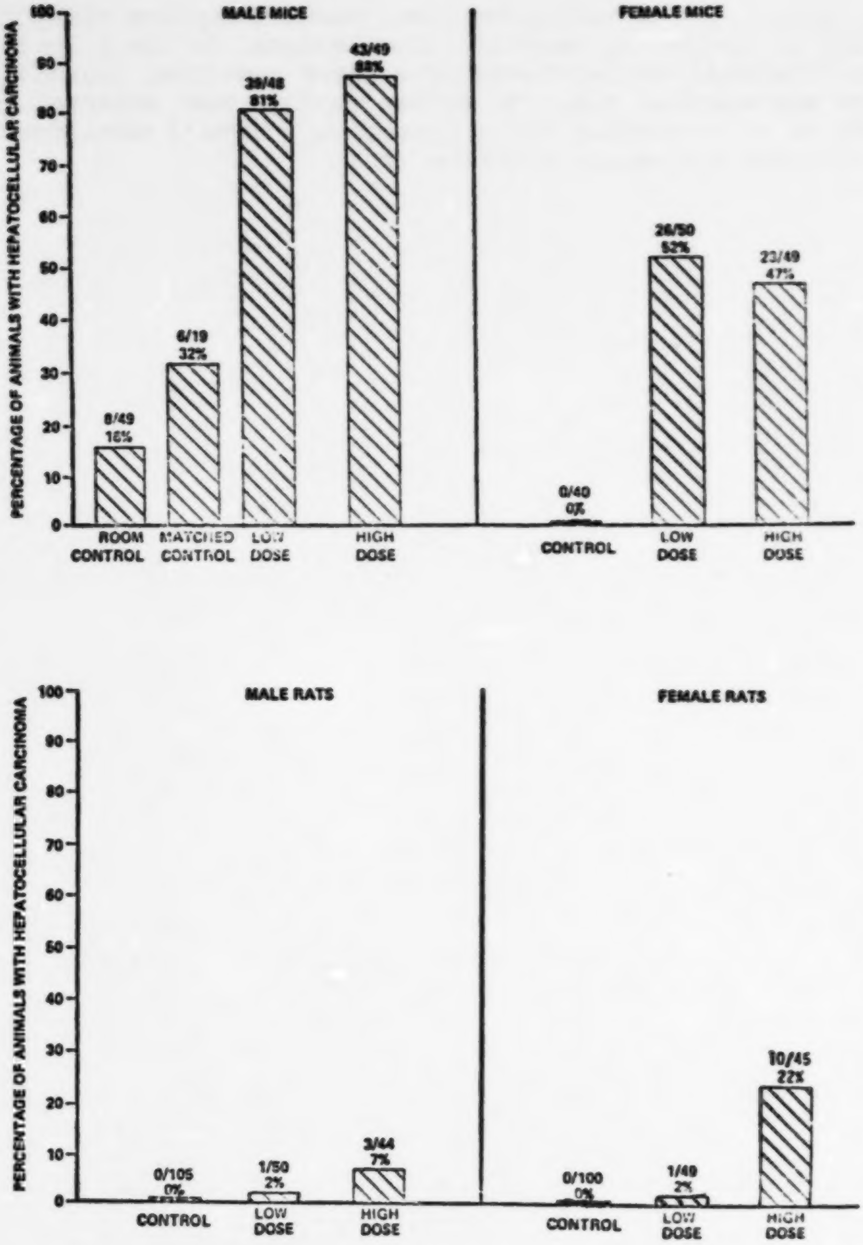


FIGURE 5.10. Comparison of incidence of heptocellular carcinoma in mice and rats following treatment with Kepone at two levels.

Source: U.S. Department of Health, Education and Welfare (1976).

The method of conducting the test, however, may have minimized the possibility of discovering hepatocellular lesions. In the Allied test, mice were numbered and survivors of a given numerical sequence were sacrificed and examined. Also, the smaller survival than observed in the NCI study at corresponding doses (including controls) means that fewer animals survived long enough to develop cancer.

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6.0 EFFECTS ON HUMANS

6.1 SUMMARY

Human exposure to Kepone and/or mirex has been documented through somewhat limited monitoring programs, epidemiologic and medical surveillance of workers engaged in the manufacture of Kepone, a systematic epidemologic survey of a community where Kepone was manufactured, and analytical confirmation of residues of both pesticides in human tissues and man's food chain.

Potential sources of exposure of humans to mirex are believed to be related to mirex bait application in fire ant control programs. In general, positive identification of mirex in foods and human tissue samples coincides with the areas of heaviest mirex usage. While it is probable that the primary exposure mode in general populations is through diet, there is insufficient information on the mechanisms of transfer of mirex in the environment to rule out the possibility that exposures via air, water, or dermal routes may be important. Where possible, detailed investigations of the habits (diet, occupation, etc.) of individuals submitting positive specimens would aid greatly in definitely establishing the sources as well as the mechanisms of exposure.

The extreme resistance of mirex to degradation and metabolism indicates the potential for environmental stability and biomagnification. Not unexpectedly, mirex residues in humans have been found in a survey of adipose tissue. To date, approximately 284 samples of adipose tissue of persons from areas of the U.S. treated for fire ant control have been analyzed and about 17 perecent of them had quantifiable levels of mirex. Trace amounts have been found in an additional 2 percent of the samples. The mirex concentrations ranged between trace and 1.3 ppm on a wet basis. Generally, those areas having the highest percentages of tissues positive for mirex corresponded to the areas having a history of the heaviest mirex usage.

The five main potential sources of human exposure to Kepone are: (1) household use of ant and roach baits containing Kepone, (2) occupational exposures, i.e., workers employed in Kepone production or agricultural workers who apply the insecticide to banana and tobacco crops, (3) accidental ingestion of agricultural formulations, (4) contaminated soil, water, or air, and (5) contaminated food.

Of 298 samples of human mothers' milk gathered in the southeastern U.S. by the Environmental Protection Agency during FY 1977, 9 showed Kepone levels ranging from less than 1 ppb to 5.8 ppb. EPA stated that it is uncertain at this time what hazards, if any, are posed by these levels.

The Health Effects Research Laboratory (HERL) of the Environmental Protection Agency has reported the results of two preliminary studies on Kepone levels found in human blood and sebum from residents of the Hopewell, Virginia, area. The Kepone source was traced to Life Sciences Products Company (LSPC), the nation's sole manufacturer of Kepone. A high blood Kepone level (7.5 ppm) of a worker led to a detailed investigation of present and former LSPC employees.

Between March, 1975, and July, 1976, interviews, examinations, and blood specimens were obtained from all 33 current employees and 100 former workers (a coverage of 90 percent of all persons who had worked at LSPC). These data indicated that of the 133 workers studied, 76 had contracted an illness chemically compatible with Kepone toxicity, 49 had neurological symptoms with or without subjective symptoms, and 27 had subjective symptoms only. Evidence of symptoms was much higher among production personnel (64 percent) than among other employees not directly involved in production (16 percent).

Studies by Guzelian, et al., at the Medical College of Virginia suggest that symptoms of toxicity in LSPC employees were observable as early as 1 week after beginning work and persisted after termination of exposure up until the time of examination. Of the LSPC employees examined, 17 were symptomatic and had whole blood Kepone concentrations ranging from 1.5 to 26.0 ppm with a mean of 8.3 ppm. Blood assay of the asymptomatic group showed a 1.8 ppm mean concentration of Kepone. Since over 50 percent of these workers had blood levels in the lower range of values for the symptomatic group, blood levels cannot be considered a sensitive diagnostic test in this population. Lower blood levels typically seen in these persons may reflect removal of Kepone from the blood to storage areas in body fat or liver. High hepatic concentrations of Kepone suggest that: (1) the pesticide may undergo biotransformation in the liver, and (2) transport of Kepone and its metabolites into bile could serve as important routes of excretion. The most pronounced physiological effects of Kepone intoxication are neurological impairment, significant hepatomegaly, and dysfunction of the endocrine and metabolic systems, the latter two resulting in sterility, weight loss, and irritability.

Therapy with selected ion exchange resins which are capable of precipitating Kepone from bile may be efficacious in treatment of Kepone poisoning, particularly if administered soon after Kepone ingestion.

A systematic survey by HERL to establish the geographic limits of the Kepone exposure area in Hopewell showed that of the 216 blood samples analyzed, 40 (approximately 19 percent) contained 5 to 50 ppm Kepone. Thirty-four of these 40 samples were from people in the residential area within 400 meters of LSPC. Two additional samples having detectable Kepone were from people living in the apartment building to the north of

LSPC, and the remaining four were from people residing 800 or more meters from the plant. The remaining 176 samples contained a nondetectable amount (less than 5 ppb) of Kepone.

Human sebum samples which were collected from hospitalized workers from LSPC contained 0.2 to 3.0 micrograms of Kepone whereas there was no detectable Kepone in samples from the general population. Sebum from some of the nonhospitalized workers contained 0.05 to 0.8 micrograms of Kepone.

The established tolerances for mirex are 0.1 ppm (negligible residue) in the fat of meat of cattle, goats, hogs, horses, poultry, and sheep; 0.1 ppm (negligible residue) in milk fat and eggs; and 0.01 ppm (negligible residue) in or on all other raw agricultural commodities.

In order to determine the fate of mirex in cattle grazing on treated pastures, the U.S. Department of Agriculture analyzed 1000 samples of subacutaneous fat from 5 different locations on the carcasses. Residues were found in 88 percent of the samples with levels ranging from 0.0001 to 0.125 ppm, and an average value of 0.026 ppm. Only one sample, 0.125 ppm, contained in excess of the established tolerance of 0.1 ppm for mirex in the fat of beef cattle. Milk from cattle in treated areas may contain minute quantities of mirex; but the amounts are at least 100 times lower than the presently established levels for milk.

From the large reservoir of fish data which have been reported concerning mirex in Lake Ontario and its tributaries, the U.S. Food and Drug Administration (FDA) found that a significant number of species of fish exceed the present FDA standard of 0.1 ppm. Fish with high mirex concentrations also had high PCB levels.

FDA's surveillance of South Atlantic and Gulf Coast states indicated that none of the assays showed detectable mirex in shellfish and crustaceans in Alabama, Louisiana, Georgia, northeast Florida, Mississippi, and South Carolina. In the case of finfish, however, all surveys except the Texas survey revealed some samples with detectable mirex levels (in Alabama, Arkansas, Louisiana, and Mississippi).

Kepone has been found in freshwater trout (0.15 to 0.17 ppm) and suckers (0.18 ppm) taken from Spring Creek, which runs near the Nease Chemical Plant, State College, Pennsylvania, a former producer of Kepone (1958-59 and 1963) and mirex, as late as 1974.

The observation of high levels of Kepone (0.02 to 14.4 ppm) in fish, shellfish, and crabs from the Hopewell, Virginia, area, together with the findings of high levels of Kepone (1.4 ppm) in sediments from various James River locations, prompted an EPA ban (December 17,1975) on taking fish, shellfish, and crabs from the James River and its tributaries from Richmond to Chesapeake Bay.

EPA has recommended to the FDA the following "action" levels: 0.3 ppm of Kepone in the edible portion of shellfish (oysters and clams), 0.1

ppm in finfish, and 0.4 ppm in crabs. These values are intended to serve as temporary levels of pesticide residues to be used as enforcement guides. EPA has also recommended a 0.03 ppm action level in processed oyster stew. Under usual action level procedures, the highest amount of a toxic chemical found not to cause chronic toxic effects in test animals is established, and reduced by a factor of 1/100. However, because of public concern with Kepone, a safety factor of at least 1/1000 was used.

Control of the banana root borer comprised the only food or feed use of Kepone. Original tolerance (now revoked) was 0.01 ppm in or on the fruit, although evidence submitted with the registration petition indicated that residues, if any, in the edible pulp would be less than the lowest detection level (0.005 ppm).

The possible carryover from soils to other crops of Kepone used to control wireworms in tobacco fields has not been investigated.

This section focuses on the epidemiology and toxicology of human exposure to mirex and Kepone. The doses to the U.S. population in general and to specific subgroups potentially most at risk have been estimated in another recent study (Suta, 1977) based in part on the findings of this investigation.

6.2 SOURCES OF POTENTIAL EXPOSURE TO MIREX

Data obtained from the U.S. Department of Agriculture (Personal communication, 1977) show that approximately 186,000 kg (410,100 lb) of mirex were applied between 1962 and 1973 in Federal-State cooperative programs to control the imported fire ant (Table 2.4). The heaviest usage has occurred in the states of Alabama, Florida, Georgia, Louisiana and Mississippi; mirex has also been used in lesser amounts in Arkansas, North Carolina, South Carolina and Tennessee. Its resistance to chemical, thermal and biochemical degradation along with evidence from animal studies suggest that mirex may be highly bioaccumulative. Sampling programs involving analysis of human tissue and foods have been initiated to define the extent of human exposure. In general, positive identification of mirex in foods and human tissue samples coincide with the areas of heaviest mirex usage. Surveys of wildlife conducted in areas most heavily treated with mirex have revealed highest concentrations of mirex in predatory fish, birds and game, which if consumed by man, could represent important sources of exposure to mirex. However, levels of mirex in commercially raised food products, on the other hand, have usually been well below the established tolerances.

Little information was found on air, water or dermal mirex exposures. With the exception of persons directly involved in manufacture or application of the product, the contribution of these sources to total exposure is probably minimal; however, the importance of these potential exposure routes cannot be definitely ruled out.

Currently available data are inadequate for a thorough appraisal of human exposure to mirex. Three very essential problems preclude such an assessment:

(1) Presently, the fate of mirex once it enters the environment cannot adequately be specified; information on the mechanisms of transfer through the environment is lacking.

(2) Mirex is indistinguishable from the chemically identical product, Dechlorane, which is used extensively as a flame retardant. The patterns of usage and distribution of Dechlorane in the environment are unknown. Consequently, mirex residues in some cases may stem from flame-retardant uses rather than fire ant control usage.

(3) The location of application of almost half of the mirex (sold during 1962-1973) cannot be accounted for.

6.2.1 Mirex in Human Tissues

The extreme resistance of mirex to degradation and metabolism indicates the potential for environmental stability and biomagnification. Not unexpectedly, mirex residues in humans have been found in surveys of adipose tissue conducted by the National Human Monitoring Program for pesticides.

The Ecological Monitoring Branch, Technical Services Division, of the U.S. Envionmental Protection Agency operates several of the component programs of the Interagency National Pesticides Monitoring Program. The basic operation and scope of the adipose survey remain unchanged from the original program as described by Yobs (1971) except that the same human tissue samples formerly analyzed for mirex will also be analyzed for Kepone. In addition, blood serum samples obtained from the Health and Nutritional Examination Survey will also be analyzed for mirex (Anon., 1976a). Data from this program are indicative to some degree of environmental contamination and distribution of this chemical. Since the human monitoring survey operates on a national scale, the stratification by census division or region does not conform to the area in which mirex has been used. The national scope of the human monitoring survey is incompatible with precise estimations of the chemical epidemiology of mirex (Kutz and Strassman, 1976).

A special monitoring program has been designed to collect representative samples of human adipose tissue from residents of areas with a history of mirex applications to determine the incidence and residue levels of mirex. Forty collection sites scattered through the southeastern states were selected for the survey. Adipose tissue samples for this project were obtained through the co-operation of medical pathologists and examiners located in cities within the collection areas. Tissues were taken from surgical specimens previously excised for pathological examination and from postmortem examinations. Since tissues representative of the general population were requested, patients having a diagnosis of known or suspected pesticide poisoning, cachetic patients, and individuals institutionalized for long periods were excluded from the sampling. To date, approximately 284 samples of adipose tissue have been analyzed and about 17 percent of them had quantifiable levels of mirex as determined by electron capture gas chromatography (Anon., 1976b). Trace amounts have been found in an additional 2 percent of the samples. The mirex concentrations range between trace and 1.32 ppm on a wet weight basis. By state, the number of samples and the percentage positive for mirex are:

State	Number of Samples	Positive Samples, Percent
Louisiana	47	40
Mississippi	28	32
Georgia	51	24
Alabama	27	11
S. Carolina	17	6
Florida	53	6
Texas	52	0
N. Carolina	9	0

Generally, those states having the highest percentages of tissues positive for mirex corresponded to the areas having a history of the heaviest mirex usage. For example, Louisiana, Mississippi, and Georgia, which ranked first, second, and third were also the three highest states in terms of aggregate acres treated with mirex under federally assisted fire ant control programs (Table 2.4). Perhaps more relevant is the fact that these same three states had the largest number of acres treated in 1973, the most recent year for which data were available (U.S. Department of Agriculture, 1977).

6.3 SOURCES OF POTENTIAL EXPOSURE TO KEPONE

The five main potential sources of human exposure to Kepone are: (1) household use of ant and roach baits containing Kepone, (2) occupational exposures, i.e., workers employed in Kepone production or agricultural workers who apply the insecticide to banana and tobacco crops, (3) accidental ingestion of agricultural formulations (mainly by children), (4) contaminated soil, water or air, and (5) contaminated food.

The major use of Kepone in the continental United States is as a roach and ant bait in houses and on lawns and gardens. Several registrations for Kepone bait formulation enclosed or not enclosed in traps, provide for general applications along baseboards, shelves, sills, or wherever ants may appear. Label directions do not always limit the amount of Kepone bait that can be applied to a single room. Although the labels provide a warning not to apply in areas accessible to children or domestic animals, the direction to apply where ants appear could result in application in areas clearly accessible (U.S. Environmental Protection Agency, 1975a).

A search of the Pesticide Episode Review System (PERS) data file identified 56 non occupational cases of human poisoning involving the active ingredient Kepone (Davida, 1976). Of these, 52 involved children under 5 years of age, two involved adults (17 to 65), and two specified no age. Of the 52 episodes involving children under five, 43 occurred in the home. Nine did not indicate a location. All home-related episodes involved products designed to control household insect pests, primarily ants and roaches. The majority of these involved children who found the pesticide (presumably contained in bait traps) and placed it into their mouths. No symptoms were listed in any of these instances. Several reports stated that treatment rendered was precautionary rather than in response to symptoms. Of the two episodes involving adults, one was an attempted suicide and the other a job related incident in which a man who worked in a pesticide packaging plant developed dermatitis on his hands. No fatalities were reported involving the active ingredient Kepone. The only health effect noted in any of the reports was dermatitis.

Another use entailing human exposure is the use of 5 percent dust on banana plants in Puerto Rico. As noted on the Candidate for Rebuttable Presumption Against Registration (CRPAR) checklist (U.S. Environmental Protection Agency, 1976b) there is only one registered product having this use. Directions call for surface application of 8 lb of active ingredient per acre and allow for application at 6 month intervals. The registered product label specifying this use prescribes a respirator for workers. Thus, the hazard posed by this use depends upon the degree of compliance with label directions. The direct hazard to humans would be a chronic dermal or inhalation effect.

Data on environmental levels of Kepone are available only from heavily contaminated areas; the hazards to general populations outside the contaminated areas are discussed in Section 7. Low levels of Kepone excreted into human milk are suggestive of the possibility of generalized sources of exposure; however, these have not yet been traced (See Section 6.4.1.)

6.3.1 Kepone in Human Tissues and Fluids

Recent tests by the Environmental Protection Agency have uncovered minute amounts of Kepone in samples of human mother's milk taken in the southeastern United States (Anon, 1976c). Of 298 samples gathered by EPA during FY 1976, nine showed Kepone levels ranging from less than 1 ppb to 5.8 ppb. EPA said it was uncertain at this time what hazards, if any, are posed by these levels. The nine milk samples were taken from women residing in Atlanta, Georgia; Whittier, North Carolina, and five Alabama communities: Birmingham, Cullman, Decatur, Huntsville, and Mobile. Milk sampling was also conducted in six other states--Arkansas, Florida, Louisiana, Mississippi, South Carolina, and Texas--but no Kepone was found in these areas.

It is unknown whether mirex is the precursor of the Kepone in the milk samples. EPA will conduct tests to see whether it can confirm USDA's findings on the connection between the pesticides. The agency is also conducting background checks on the women with positive results to determine whether the Kepone may have come from sources other than mirex.

Results from the National Pesticides Monitoring Program on Kepone levels in human tissues have not yet been reported; information from areas having no history of mirex application should help clarify whether mirex is the source of Kepone in milk and tissue samples.

6.4 ACUTE EXPOSURE TO KEPONE

The Health Effects Research Laboratory (HERL) of the U.S. Environmental Protection Agency has reported the results of two preliminary studies on Kepone levels found in human blood and sebum from residents of the Hopewell, Virginia, area (U.S. Environmental Protection Agency, 1975b and 1976a).

On August 7, 1975, EPA was first alerted to the possibility of a pesticide exposure problem in Hopewell when a worker at the Life Sciences Products Company (LSPC), the nation's sole manufacturer of Kepone, experienced neurological symptoms and contacted a local internist (U.S. Environmental Protection Agency, 1975b). Recognizing the possibility of an occupational exposure, a blood sample of the worker was submitted for analysis. When a high Kepone level of 7.5 ppm was found in the blood sample the patient was immediately admitted to the Medical College of Virginia Hospital, and the Virginia State Health Department was informed. With the aid of the Center for Disease Control in Atlanta, Georgia, an investigation of present and former employees of LSPC was promptly begun.

6.4.1 Epidemiology of Kepone Poisoning

Between March, 1974, and July, 1975, 76 (57 percent) of the workers at Life Sciences Products Company (LSPC) contracted an illness clinically compatible with Kepone toxicity. The principal manifestations of the disease were nervousness, tremor, weight loss, opsoclonus, (erratic or "jumpy" eye oscillations), and pleuritic chest pain. As shown in Table 6.1 workers exhibited a syndrome closely resembling that seen in animals suffering from Kepone exposure (Center for Disease Control, 1976).

Of a total of 148 persons who had worked at LSPC since it opened, 33 were employed at the time the plant closed. Interviews, examinations, and blood specimens were obtained from all 33 current employees and 100 of the 115 former workers, representing a coverage of 90 percent of all persons who had worked at LSPC. Of these 133 workers studied, 76 were affected; 49 had a neurologic abnormality with or without subjective symptoms and 27 had subjective symptoms only. Attack rates were much higher among production personnel (64 percent). Rates of Kepone poisoning are classified according to job category in Table 6.2.

TABLE 6.1. SUMMARY OF TOXIC EFFECTS OF KEPONE IN HUMANS AND ANIMALS[a]

Animals	Humans
Excitability	Nervousness
Tremor	Tremor, ataxia
Loss of weight	Loss of weight
Dermatologic changes	Skin rash
Testicular atrophy	Sterility
Increased liver-to-body weight ratio	Abnormal liver function
Increase in hepatocellular	Opsoclonus
	Joint pain
	Pleuritic pain

[a] Adapted from Center for Disease Control, (1976).

TABLE 6.2. KEPONE POISONING ATTACK RATES BY JOB CATEGORY[a]

	Current Workers			Former Workers			Total Workers		
	Cases	Total	Attack Rate, percent	Cases	Total	Attack Rate, percent	Cases	Total	Attack Rate, percent
Secretarial/Office	0	5	0	0	4	0	0	9	0
Janitorial	2	4	50	1	6	17	3	10	30
Maintenance	4	7	57	14	28	50	18	35	51
Pad Men	1	1	100	3	6	50	4	7	57
Lead Operators/ Foremen	7	9	78	9	13	68	16	22	73
Dryer and Filler Operators	6	7	86	29	43	67	35	50	70
Total	20	33	61	56	100	56	76	133	57
				Nonproduction Workers			3	19	16
				Production Workers			73	114	64
				Total			76	133	57

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[a] Adapted from Center for Disease Control (1976).

Limited ambient air measurements have been made in the LSPC facility but are insufficient to adequately define exposure levels of Kepone. The data available indicate that levels probably were in the range of 0.016 to 3.0 mg/m^3. Workers involved in the drying operations and presumably exposed to 3.0 mg/m^3 levels had blood Kepone levels of 5 to 60 ppm. These workers had a 65 percent attack rate. This compares with a mean level of Kepone of 8.48 ppm measured in the blood of 7 of the 10 workers actively employed and having signs of Kepone poisoning at the time the survey was conducted. The remaining three asymptomatic individuals had mean blood Kepone levels of 1.57 ppm.

Table 6.3 shows the ranges and means of blood Kepone levels for various groups tested. Data on Hopewell area residents come from a community survey more fully discussed in Section 6.4.4 and are included here for comparative purposes only. Interpretation of blood Kepone levels and symptoms for the groups of exposed individuals other than LSPC workers is difficult. Blood Kepone levels of these persons were much lower than those of LSPC workers, and their exposure was much less certain. Family members were exposed either by general air pollution in the vicinity of the plant or through dust carried home on the clothing of workers. Sewage-treatment-plant workers were exposed through work on the Hopewell sewer system that had been contaminated with Kepone. Cab drivers were exposed through dust on the clothing of workers whom they drove home from work.

The workers for Allied Chemical Corporation who had been employed in earlier Kepone production at the Allied semiworks plants present an interesting comparison group. They were occupationally exposed to Kepone and would be expected to have health problems analogous to the LSPC workers; however, several factors were different. Kepone was produced only a few months each year at Allied rather than continuously; workers described a much cleaner operation at Allied than at LSPC; and there appeared to have been far less employee illness at the Allied plant than at the LSPC plant. Five of the 39 Allied workers who were exposed at least 16 months before blood sampling exhibited possible Kepone toxicity; of these five, four demonstrated a neurologic abnormality consistent with Kepone toxicity, and one of them complained of a complex of systems consistent with Kepone toxicity. However, at the time of examination three had nondetectable blood Kepone levels, and the other two had levels of only 0.009 ppm and 0.02 ppm. Blood levels in these Allied workers might be low or nondetectable because Kepone had been stored in toxic concentrations in other parts of the body (fat,brain, and liver) or excreted from the body after causing residual tissue damage (Center for Disease Control, 1976).

6.4.2 Clinical Findings in LSPC Workers

Understanding the clinical manifestations of Kepone toxicity and of instituting rational therapy has been hampered by the lack of precedent in humans and because little information on the topics of Kepone

TABLE 6.3. BLOOD KEPONE LEVELS BY GROUPS OF EXPOSED PERSONS[a]

Group	Number Tested	Number With Detectable Levels	Percent With Detectable Levels	Range of Detectable Levels, ppm	Means of Detectable Levels, ppm
Affected LSPC workers	57	57	100	0.009-11.8	2.53
Unaffected LSPC workers	49	48	99	0.003- 4.1	0.60
Family members of LSPC workers	32	30	94	0.003- 0.39	0.10
Allied Chemical Kepone workers	39	30	77	0.003- 0.45	0.06
Neighborhood workers	32	23	72	0.003- 0.031	0.011
Sewage treatment plant workers	10	6	60	0.004- 0.014	0.008
Cab drivers	5	1	20	0.003	0.003
Truck drivers	2	1	50	0.004	0.004
Hopewell residents	214	40	19	0.005- 0.0325	0.011

[a] Adapted from Center for Disease Control (1976).

distribution, biotransformation and excretion is available from animal studies.

The only information on the metabolic characteristics and effects of Kepone in humans comes from studies of exposed workers at Hopewell, Virginia. As part of an ongoing study of the toxicity and pharmacokinetics of Kepone at the Medical College of Virginia, Guzelian, et al., (1975) have reported clinical findings of 32 employees of LSPC who were examined for symptoms and signs of Kepone associated toxicity. The period of exposure of affected workers ranged from 3 to 18 months, with symptoms often appearing as early as 1 week after beginning work at LSPC and persisting after termination of exposure up until the time of the examination.

6.4.2.1 Blood Levels--

Of the LSPC employees examined, 17 were symptomatic and had whole blood Kepone concentrations ranging from 1.5 to 26.0 ppm with a mean of 8.3 ppm. In the asymptomatic group, the mean concentration of Kepone in the blood was 1.78 ppm. Since over 50 percent of these workers had blood levels in the lower range of the values for the symptomatic group, blood levels are not considered a sensitive diagnostic test in this population. Similar examinations of 81 former employees identified 33 additional suspected cases of Kepone toxicity. Significant amounts of Kepone have been found in the blood of family members and even household pets of some of the affected workers.

Results of a similar analysis of blood specimens from workers at LSPC were also reported by the Center of Disease Control Toxicology Branch (Center for Disease Control, 1976). The blood Kepone levels observed in the workers exhibiting signs of toxicity ranged from 0.009 to 11.8 ppm, whereas those for asymptomatic workers ranged from nondetectable to 4.1 ppm. The mean Kepone blood level in poisoned workers was significantly different from that of persons in the non poisoned category: 2.53 versus 0.60 ppm, respectively. Several persons with neurologic symptoms had relatively low blood levels, however.

6.4.2.2 Tissue Levels--

The CDC does not feel that Kepone levels in blood accurately reflect body burden or potential for toxicity, especially among former workers no longer exposed to Kepone, since lower blood levels typically seen in these persons may reflect removal of Kepone from the blood to storage areas in body fat or liver. Also, in persons who continue to manifest neurologic symptoms without continued exposure, the Kepone level may have dropped below the limit of detection in the blood, but Kepone may have caused irreversible damage or may still be present in toxic concentration in the brain or other organs. Table 6.4 reveals the inconsistency in blood and tissue levels in severely affected workers, illustrating the inability of blood levels to reflect the full body burden of Kepone.

TABLE 6.4. KEPONE LEVELS IN VARIOUS TISSUES OF KEPONE WORKERS[a]

	Kepone Concentration[b], ppm					
Patient	Serum	Liver	Fat	Urine	Cerebrospinal Fluid	Saliva
1	0.4	5.3	--	0.015	--	0.125
2	5.6	81.4	--	0.020	0.028	1.49
3	6.0	173.0	50.0	--	0.021	--
4	9.45	81.0	52.0	--	0.027	0.54

[a] Adapted from Center for Disease Control (1976).

[b] Analyses performed by Consolidated Laboratory Services, Virginia State Department of Health.

6.4.2.3 Metabolism of Kepone--

Studies at the Medical College of Virginia have shown not only a wide range of blood Kepone concentrations among the hospitalized workers, but also considerable fluctuations of blood Kepone levels within individuals. One possible explanation of this variability may be that the small quantity of Kepone present in extracellular fluids equilibrates with the bulk of the highly lipophilic compound which is sequestered in tissue lipids and membranes. Higher concentrations of Kepone in liver and fat than in blood, shown by this preliminary study (Guzelian, et al., 1975), may imply that Kepone is stored preferentially in tissue reservoirs. The high hepatic concentration of Kepone suggests that the pesticide may undergo biotransformation in the liver and that transport of Kepone and its metabolites into bile could serve as important routes of excretion. Consistent with this hypothesis, Kepone has been detected in both the feces and bile of the hospitalized patients.

The extent of biotransformation of Kepone in humans remains unanswered. Dr. R. V. Blanke at the Medical College of Virginia (cited in Guzelian, et al., 1975) has found unidentified halogenated hydrocarbon compounds other than Kepone in gas chromatographic analysis of blood and urine. These findings are equivocal concerning Kepone metabolism, however, because these compounds could either represent products of Kepone metabolism or could be related to the halogen-containing antianxiety benzodiazepine drugs that many of the patients were taking at the time of sampling. Among patients who received no halogenated drugs, the unidentified compounds were not detectable in blood, urine, or liver.

6.4.2.4 Effects--

The most pronounced physiological effects of Kepone intoxication relate to the neurologic systems, the liver and the endocrine system.

6.4.2.4.1 Neurological effects--The most prominent symptom in the great majority of the patients was neurological impairment. Tremors, jumpy eye movement (opsoclonus), exaggerated startle response, and various forms of cerebellar dysfunction were the most typical neurologic manifestations (Guzelian, et al., 1975). Results of electromyography and nerve conduction studies were normal in most cases, although there was some evidence of neuropathy and myopathy. Electron microscopy examination gave evidence of peripheral neuropathy, and deposits of unidentified material were present in some gastrocnemius and sural nerve biopsies. The nature of these ultrastructural changes is discussed in detail by Martinez, et al., (1976).

6.4.2.4.2 Hepatotoxic effects--Although a majority of patients had significant hepatomegaly, the extent of liver involvement in these patients was generally mild. Signs of acute or chronic liver failure or portal hypertension have not been observed. A two-to threefold elevation of alkaline phosphatase was present in about one-third of the patients while the other liver-function tests typically yielded normal results.

Liver histology in 12 patients revealed increased fat, numerous dense bodies and a proliferation of smooth endoplasmic reticulum.

6.4.2.4.3 Reproductive effects--Sterility was the most striking abnormality of the endocrine and metabolic system. Anderson, et al., (1976) hypothesized that the underlying cause of infertility may be maturation arrest (which has been associated with administration of organic pesticides in animals). Anderson, et al., (1976) examined 16 LSPC workers who exhibited evidence of neurologic hepatic and testicular dysfunction from prolonged exposure to Kepone. Sperm counts obtained in seven patients revealed azospermia in one previously fertile patient and counts of less than 20 million in five others. All seven had less than 20 perrcent motility and increased abnormal forms. Three of these patients also have abnormally small testes, and testicular biopsies revealed a generalized maturation arrest. Although eight of the sixteen patients had decreased libido, testosterone levels were normal in five patients and elevated in two. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were found to be normal. Blood Kepone levels and sperm counts correlated poorly, however, such a finding was not unexpected in view of the poor correlation between blood and tissue Kepone levels (Anderson, et al., 1976).

Prompted by the findings of infertility and dramatic weight loss in the presence of normal or increased food intake, Guzelian, et al., (1975) conducted a detailed investigation of endocrine and other metabolic functions of the Kepone poisoned workers, discussed in the following section. The authors have speculated that the features of restlessness, irritability, tremulousness and weight loss in the absence of evidence of malabsorption or decreased appetite might be accounted for by the presence of a "hypermetabolic" state.

6.4.2.5 Treatment--

One study by Cohn, et al., (1976), suggests that therapy with resins (i.e., cholestryamine) which are capable of precipitating Kepone from bile may be efficacious in treatment of Kepone poisoning. It appears that cholestryamine facilitates excretion by inhibition of intestinal biotransformation or prevention of reabsorption of Kepone in the intestine following excretion into the bile.

The findings of a preliminary study of 32 hospitalized workers poisoned after chronic exposure to large quantities of Kepone have been reported by Cohn, et al., (1976). Significant findings include: (1) abnormal liver histology, specifically increased fat, dense bodies and proliferation of smooth endoplasmic reticulum; (2) high concentrations of Kepone in liver, whole blood and subcutaneous fat as shown in Table 6.5. (3) a partition of Kepone between fat and blood (mean = 7.4:1) 10 to 20 times lower than that reported for other lipophilic chlorinated hydrocarbons; (4) failure to identify metabolites of Kepone in tissue, urine, or feces; (5) negligible excretion in urine and sweat; (6) the principal route of excretion of Kepone is the feces.

TABLE 6.5. TISSUE DISTRIBUTION OF KEPONE IN HOSPITALIZED WORKERS[a]

Tissue	Number of Samples	Kepone Concentration, ppm	
		Mean	Range
Liver	10	75.9	13.3-173.0
Whole blood	32	5.8	6.6-32.0
Subcutaneous fat	16	21.5	2.2-62.0

a Source: Cohn, et al., (1978). Reprinted, with permission from the New England Journal of Medicine. (c) The Massachusetts Medical Society (1978)

Kepone was eliminated in the feces of 15 patients at a mean daily rate of 0.075 percent (range 0.019 to 0.279 percent) of the estimated total stores of Kepone. However, fecal excretion accounted for only 10 percent of biliary excretion of Kepone, as estimated from samples of duodenal drainage. These differences suggest that Kepone may be converted in the intestine to unmeasured chemical species and/or may undergo enterohepatic circulation. The latter hypothesis was evaluated in a clinical trial of cholestryamine therapy recently reported by Cohn, et al., (1978).

Approximately 12 months after their initial evaluation, 29 of the original group of 32 LSPC workers participated in a randomized trial of oral cholestryamine therapy. The men were stratified into three groups according to blood Kepone level at entry to the study. The "high" group consisted of 10 men having blood Kepone levels of grater than 1000 ng/liter (10 ppm). "Medium" and "low" groups consisted of ten men having blood Kepone levels of 5-10 ppm and nine men having blood Kepone levels of 1-5 ppm, respectively. Within each of these groups, the men were randomly allocated to treatment with either cholestryamine or a placebo.

Patients were treated for 5 months (placebo or cholestryamine, 36 grams per day). The participants were examined biweekly and samples were taken of blood, subcutaneous fat and urine. The drug was well tolerated and in 11 of the 12 patients treated with cholestryamine, an accelerated rate of disappearance of Kepone from the blood was observed relative to each patients rate during the pretreatment "control" period. The average half-life of Kepone in the blood for patients given cholestryamine was 80± 4 days, a significant reduction compared to 165 ± 27 days observed in the control period. On the other hand, the average half-life of patients treated with a placebo was unchanged from their pretreatment values.

Cholestryamine had a dramatic effect in stimulating the rate of Kepone excretion in the stool as shown in Figure 6.1. (Control collections of stool were obtained from each patient for at least 72 hours. Treatment with cholestryamine was started, and 48 hours later, stool was collected for a second 72-hour period).

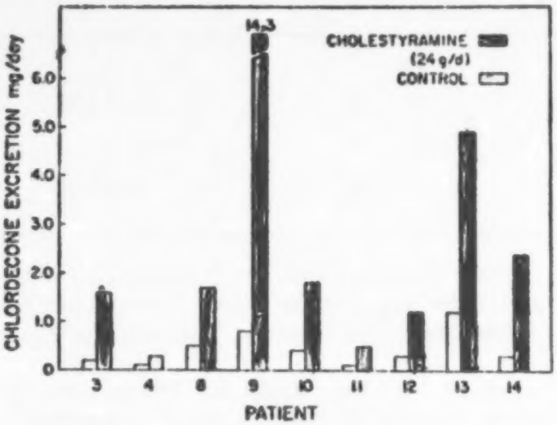

Figure 6.1. Stimulatory effect of cholestryamine on the rate of excretion of chlordecone in the stool.

Source: Cohn: et al. Reprinted, with permission, from the New England Journal of Medicine. (c) The Massachusetts Medical Society (1978).

The decrease in blood Kepone levels was accompanied by a decrease in the severity of neurologic symptoms in many of the patients. At the outset of observation, 11 of 22 patients were unable to work due to the severity of their neurological disorders whereas after completion of the clinical trial, only three remained severely impaired.

Sterility or extreme depression in sperm count is another toxic response to Kepone. Interestingly, most of the patients showed improvement in this symptom. Motile sperm counts increased in 12 of 13 patients as blood Kepone levels declined.

The study also provided some preliminary evidence that there may be some biotransformation of Kepone, possibly occurring in the liver or intestine. Cohn, et al., (1978) reported the finding of an unspecified chlordecone alcohol in the human stool. This alcohol is present in the stool in concentrations equal or greate than chlordecone (Kepone).

6.4.3 Risk Assessment of Carcinogenic Effects

The EPA Carcinogen Assessment Group headed by E. L. Anderson has performed a risk extrapolation for the possible carcinogenic effects of allowing existing stocks of Kepone to be used up (U.S.Environmental Protection Agency, 1976c). Only a linear extrapolation was possible with the limited exposure data available. Use of nonlinear extrapolation methods requires knowledge not only of average exposure but also of the distribution around the average. This is a particular problem in the present case where accidental ingestion by children may be a significant portion of total human Kepone exposure. The risk assessment group has derived an average daily exposure to Kepone expressed as ppm in the diet, taking into consideration the following factors: (1) the number of people exposed to Kepone through the use of existing stocks, (2) the number of years necessary to use up existing stocks (estimated at 3 years), and (3) the proportion of the Kepone stocks which will eventually result in human exposure. Existing total stocks then on hand were estimated by EPA at 537.8 lb (U.S. Environmental Protection Agency, 1976c).

The risk assessment group derived the slope of the dose response curve from NCI data for male mice fed 23 ppm of Kepone (the case which yields the highest results). The probability of an individual developing a tumor due to exposure at the calculated Kepone dose was derived under the one-hit theory of carcinogenesis. This calculation assumes that the effect of exposure to Kepone would be the same if the same dose were taken in a single accidental ingestion or over a period of several years. While this assumption is consistent with the one-hit theory, it may not be true in fact. The calculation does not take into account the special circumstance that exposure of small children to Kepone may be greater than exposure of other age groups. This could affect the results in two ways. First, small children may be more or less susceptible than other groups. Second, competing risks (the probability that a cancer is initiated, but death from another cause intervenes before it is expressed as a tumor) would be much smaller.

Because of the lack of firm exposure data, three different assumptions were made. First, the highest risk to a single individual from the worst reasonably possible exposure was estimated by assuming that a child would eat six 3-oz tubes of ant paste over 3 years. This would result in a cancer risk of 1/400, extrapolating from the animal data. The number of children exposed at this level, if any, is likely to be very small. Second, using data from Pesticide Episode Reporting System as a starting point, it was assumed that 3,750 children might each eat about half an ant trap containing Kepone. This would result an expected 0.083 cases of cancer or a probability of 1/12 of one case of cancer. Finally, an attempt was made to estimate parametrically the effects of all routes of exposure, including vaporization, spilling onto kitchen surfaces, etc., in addition to accidental ingestion by children. Using a linear no-threshold extrapolation, the resulting number of cases of cancer would be 540 multiplied by the proportion of the Kepone which reaches humans.

An estimate of probability (P) is needed to arrive at a final projection. For example, if it is estimated that 1/10 of 1 percent of the total stocks would ultimately reach people (P = 0.001), then about half a case of cancer would be projected to result (U.S. Environmental

Protection Agency, 1976c). Unfortunately, no similar risk extrapolations have been based upon the probable Kepone exposure levels and dosage received by workers at the Life Sciences Products Company.

6.4.4 Subacute Exposure to Kepone

Allegations of former employees of LSPC that substantial amounts of Kepone had been discharged into the sewage system prompted a request from the Virginia State Health Department for assistance in assessing environmental contamination (U.S. Environmental Protection Agency, 1975b). A systematic community survey was begun by EPA's Health Effects Research Laboratory (HERL) in August, 1975, to ascertain whether human exposures (as reflected by blood Kepone levels) had occurred and if so, to attempt to define the geographic limits of the exposure area.

Concentric circles with radii equivalent to 400, 800, 1200, and 1600 meters (1/4, 1/2, 3/4, and 1 mile) were mapped around LSPC. The area within 400 meters was primarily commercial except for a small, predominantly black residential area south of the plant and a high-rise apartment building to the north occupied mostly by elderly whites. Every fifth occupied residence was chosen systematically in the innermost areas while on the outer three circles, a randomly selected single unit dwelling was selected from each residential block intersecting the circle.

All occupants in each selected residence were invited to participate. A standardized questionnaire was administered including information on age, race, sex, self-reported neurologic symptomatology, and residential and occupational history of each occupant. In all, 93 housesholds were sampled yielding a total of 216 participants. A venous blood sample was drawn from each participant and a sebum sample was obtained by wiping the forehead with a 5 x 5 cm gauze pad soaked with acetone. Each blood extract was first analyzed by electron-capture gas liquid chromatography (GC) at a quantitative screening level of 1 ppb.

At Kepone levels below 5 ppb, a quantitative value could not be assigned with any degree of confidence. Therefore, the lower limit of reporting was set at 5 ppb, with samples below 5 ppb being reported as "nondetectable". Samples containing over 5 ppb were analyzed on both of the GC columns used, with selected samples subjected to further quantitative confirmation by GC.

Of the 216 blood samples analyzed, 40 (approximately 19 percent) contained 5 to 50 ppb Kepone. The remaining 176 samples contained nondetectable levels (5 ppb) of Kepone. Thirty-four of these 40 samples were from the residential area within 400 meters south of LSPC. Two additional detectable samples were from the apartment building to the north of LSPC, and the remaining four were from participants residing 800 meters or more from the plant.

Percentages of blood samples above the detectable level were:

39 percent (34/87) in the residential area south of LSPC
7.7 percent (2/26) in the apartment to the north
5.9 percent (2/24) 800 meters away
2.6 percent (1/39) 1200 meters away
3.3 percent (1/30) 1600 meters away.

None of the people reported having worked at either LSPC or at the Allied Chemical Corp. semiworks where Kepone had been manufactured prior to 1974.

Within the most exposed area there was marked bimodality, according to age, in the proportion of persons with detectable blood levels, reaching a peak in pre-teen age children and in older adults. A marked depression was observed in the intermediate age groups. None of the analyzed blood samples collected from the general population contained greater than 50 ppb of Kepone. These blood levels of Kepone are substantially lower than those found in LSPC employees. Nevertheless, they should be viewed as evidence of environmental contamination.

Human sebum samples which were collected from hospitalized workers from the Kepone manufacturing plant contained 0.2 to 3 microgram of Kepone whereas there was no detctable Kepone in samples from the general population (U.S. Environmental Protection Agency, 1975b). Sebum from some of the non hospitalized workers contained 0.05 to 0.8 microgram of Kepone.

Although these data are of diagnostic value, they merely suggest exposure routes. Little information is provided about the extent and duration of exposure, and nothing about the distribution and accumulation in target organs and tissues or metabolism or elimination of this compound can be determined on the basis of these findings. Lack of 24-hour urine samples and of fat biopsies or adipose tissue samples collected simultaneously with the blood samples during the exposure period precludes estimates of average daily doses or intake of Kepone. Owing to the lack of data relating blood levels and manifestation of adverse clinical or pathological effects, interpretation of the significance of blood levels in the 5 to 50 ppb range must be deferred. Similarly, human data on dose-response to Kepone must await the completion of the studies of Life Sciences Products Co. workers.

6.5 RESIDUES IN FOODS

6.5.1 Mirex

The established tolerances for mirex are 0.1 ppm (negligible residue) in the fat of meat of cattle, goats, hogs, horses, poultry, and sheep; 0.1 ppm (negligible residue) in milk fat and eggs; and 0.01 ppm (negligible residue) in or on all other raw agricultural commodities (U.S. Environmental Protection Agency, 1976d).

Because mirex is a very stable insecticide capable of being stored in fat, the U.S. Department of Agriculture conducted a survey to determine (1) whether cattle grazing on treated pastures would accumulate mirex in their tissues, and (2) the environmental distribution of mirex following application (Ford, et al., 1973). Samples of fat from beef cattle were obtained from two areas of Mississippi and Georgia heavily infested with fire ants, which were known from USDA records to have received blanket applicationsn of mirex at least twice in the past 3 years. State meat inspectors collected 1000 g samples of subcutaneous fat from five different locations on the carcasses. Limited samples of internal organs were also obtained. Similar samples were also obtained from cattle in control areas outside the region where mirex had been used. Analysis was performed by gas-liquid chromatography using a modification of the Armour-Burke method (Armour and Burke, 1970) to remove interference from PCB's. Residues of mirex were found in 88 percent of the samples, with levels ranging from 0.001 to 0.125 ppm and an average value of 0.026 ppm. Only one sample, 0.125 ppm, was in excess of the established tolerance level of 0.1 ppm for mirex in the fat of beef cattle. No residues were found in the other organs (Ford, et al., 1973).

Because mirex has been used as flame retardant under the name Dechlorane, samples from untreated areas in both the southeast and in other areas were obtained. The absence of mirex in samples from untreated areas, strongly indicates that residues found in samples from the treated areas were from the insecticide application. The authors concluded that residues can be expected in beef fat in treated areas following the use of mirex, but that application in the prescribed manner will keep residues below the tolerance level.

In obtaining a registration and tolerance level for mirex, Allied Chemical Corporation monitored whole milk and milk fat for mirex in a herd of cows grazing on a pasture treated with a single application of bait. They reported 0.002 to 0.007 ppm in whole milk and 0.02 to 0.13 in milk fat (Hawthorne, et al., 1974). (Presently there is a tolerance level of 0.01 ppm for whole milk and 0.1 ppm for milk fat).

Since the same residue levels were found in pretreatment and untreated control animals, Hawthorne, et al., doubted the validity of findings from the Allied Chemical study and initiated another milk survey. Inspectors familiar with dairy farms and processing plants in southeastern U.S. selected areas which had received two or more mirex treatments. After verifying that all the milk had come from cows that had grazed in the treated areas, a 1-quart sample of milk was taken from the storage tanks of each dairy or processing plant. Ten or more samples were taken from different locations in the following states: Florida, Georgia, Louisiana, Mississippi, and South Carolina. Samples were analyzed by gas chromatography with an estimated limit of detection of 0.3 ppb.

A total of 66 samples were obtained from the five states, including 6 control samples obtained from untreated areas. The remainder of the samples were from areas which had been treated 2 to 5 times. Of the 60 samples analyzed, none had detectable mirex residues at the 0.30 ppb level of detection; however, at least 26 of the samples were from areas which had received their last mirex treatment 1 year or more prior to collection. Another 26 of the samples were from areas which had been treated at least 6 months before sampling. Many samples contained small but questionable peaks at the same retention time as mirex; however, attempts to confirm these peaks by thin-layer chromatography (TLC), and fortifying and using additional columns, were inconclusive. The authors conclude that milk cattle must be receiving some small amounts of mirex, but not enough to show up in milk at the detection level of about 0.3 ppb. Mirex might also be better detected if the samples were taken closer to the time of treatment. Thus, milk from cattle in treated areas may contain minute quantities of mirex; but the amounts are at least 100 times lower than the presently established levels for milk (Hawthorne, et al., 1974).

Lane (1973) investigated the possible uses of food processing to degrade pesticidal residues in foods. Tissue samples (crawfish) and eggs (mallard ducks) contaminated with mirex were processed by (1) a commercial thermal processing procedure, (2) drastic heat treatment and (3) irradiation with ultraviolet light. Mirex residues from tissue samples processed by each of the three methods did not differ significantly from controls. Photolysis and gamma irradiation of eggs, however, demonstrated a highly significant difference among the mean residue content of controls and the means at various time intervals as irradiation proceeded. A linear relationship was seen between mirex degradation and increasing radiation dosage. Monohydro and dihydro degradation products of mirex were separated and identified by combined flame ionization gas chromatography-mass spectrometry and retention time data (see Section 2.3.2.1.).

In 1968-1969 the U.S. Department of Agriculture (USDA) conducted a study on Cat Island off the coast of Mississippi and in 1969-1970 the USDA and the U.S. Department of the Interior cooperated in a similar study. Both studies showed mirex residues ranging from 0.001 to 2 ppm in some bottom feeding fish immediately following a mirex treatment. Small amounts of residues could be detected as much as 2 years later (Markin, et al., 1974). Other studies have discovered mirex residues in commercial catches for human consumption well outside the immediate treatment area.

In August, 1975, the Food and Drug Administration (FDA) as part of its routine pesticide surveilliance activities, first learned that mirex was a chemical contaminant of Lake Ontario. EPA's Duluth Laboratories had reportedly found mirex residues in Lake Ontario several years earlier; however, FDA was not informed of these findings (Wessell, 1976, personal communication). From August to November, 1975, FDA laboratories analyzed 18 samples of fish representing 10 species taken from different parts of Lake Ontario (U.S. Department of Health, Education, and Welfare, 1976). As indicated in Table 6.6 all but two of the samples were found to contain mirex residues ranging from trace (less than 0.03 ppm) to 0.53

TABLE 6.6. PESTICIDE RESIDUES IN LAKE ONTARIO FISH COLLECTED IN AUGUST AND SEPTEMBER, 1975[a]

Lake Ontario Source	Specie	Residue, ppm							
		Mirex	PCB Aroclor 1254	PCB Aroclor 1248	DDE	DDT	TDE	Dieldrin	BHC
Chaumont Bay	Bullhead	T	0.39	--	0.03	--	0.01	T	T
Chaumont Bay	Bullhead	0.03	0.50	--	0.04	--	0.01	T	T
Chaumont Bay	Bullhead	0.04	0.75	--	0.05	--	0.02	T	T
Chaumont Bay	Rock Bass	T	0.55	--	0.02	--	T	--	T
Chaumont Bay	Smallmouth Black Bass	0.19	2.69	--	0.48	0.10	0.07	0.01	T
Chaumont Bay	Smallmouth Black Bass	T	1.15	--	0.09	--	--	T	T
Chaumont Bay	Northern Pike	0.08	1.60	--	0.18	--	--	--	0.01
Chaumont Bay	Northern Pike	0.06	1.29	--	0.18	--	--	--	--
Chaumont Bay	Northern Pike	0.06	1.09	--	0.13	--	--	--	0.01
Chaumont Bay	Northern Pike	0.04	0.96	--	0.13	--	--	--	--
Chaumont Bay	White Perch	0.05	1.00	--	0.19	0.06	0.06	0.19	T
Chaumont Bay	White Perch	0.12	2.94	0.43	0.42	--	0.25	0.04	--
Chaumont Bay	Yellow Perch	--	0.43	--	0.04	--	T	T	T
Chaumont Bay	Herring[b] (Mooney)	--	0.99	--	0.16	--	T	0.02	--
Salmon River Outlet Pulaski, N.Y.	Coho Salmon	0.35	10.75	--	1.11	0.22	0.25	0.10	0.15
Galloo Island area	White Perch	T	2.33	0.54	0.32	T	0.13	0.06	T
Unknown Source	Lake Trout	0.53	14.62	--	0.96	0.25	0.34	0.24	0.29
Unknown Source	Weak Fish	T	0.57	--	--	--	--	--	--

[a] Source: Food and Drug Administration, (1976).

[b] Herring sample collected by FDA; all others collected by New York State.

[c] T = trace.

ppm. The samples contained not only mirex but also varying combinations of PCB's (primarily Aroclor 1254) dieldrin, BHC, and DDT-related compounds, The accuracy of these results has been questioned because mirex was not separated from the PCB's prior to determination by electron capture gas chromatography. Also, PCB (especially Aroclor 1254) was overwhelmingly the highest residue in all samples. Since PCB has a late eluting GC peak which closesly resembles that of mirex, improper identification could result in serious errors in mirex residue calculations. Nevertheless, the authors conclude that the mirex residues reported probably do not represent spurious findings due to analytical errors because mirex residues have been confirmed in fish and fish eating birds (herring gulls) and their eggs in Lake Ontario (Kaiser, 1974; Canadian Ministry of the Environment, 1976; Alexander, personal communication, 1976).

As a result of the above findings, FDA requested and received from EPA's Office of Pesticide Programs a recommended action level of 0.1 ppm for mirex residues in fish. This action level was put into effect May 18, 1976.

Table 6.7 presents some recent mirex findings for fish in Lake Ontario and tributaries (New York State Department of Environmental Conservation, 1976). From the large reservoir of fish data presented in Table 6.7 it can be seen that a significant number of species of fish exceed the present FDA standard of 0.1 ppm. These can be summarized as follows:

Classes of fish consistently exceeding 0.1 ppm mirex

- Salmonidae (trouts)*
- Osmeridae (smelts).

Classes of fish consistently below 0.1 ppm mirex

- Esocidae (pikes)
- Cyprinidae (minnows and carps)
- Catostomidae (suckers)
- Pecidae (perches).

Classes of fish with mirex concentrations varying above and below 0.1 ppm

- Percichthyidae (temperature basses)**
- Ictaluridae (freshwater catfishes)
- Centrarchidae (sunfishes)***
- Anguillidae (freshwater eels)
- Clupeidae (herrings)

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* Except for rainbow trout

** Tended to exceed or approach 0.1 ppm except for wall eye

*** Tends to be below 0.1 ppm for smallmouth bass

TABLE 6.7. MIREX DATA FOR FISH FROM LAKE ONTARIO AND TRIBUTARIES, AS OF SEPTEMBER 22, 1976[a]

Fish				Number		Mirex	Mean
			Number	of	Concentrations,ppm		Total PCB Concentration,
Species	Family[b]	Location[c]	Analyzed	Analyses	Mean	Range	ppm
White Perch	P	NS	3	3	0.38	0.25-0.50	NR
Lake Trout	S	SS	2	2	0.35	0.28-0.42	17.4
Catfish	I	NS	3	3	0.27	0.-0-0.38	NR
Coho Salmon	S	NS	79	79	0.23	0.04-0.40	NR
Brown Trout	S	NS	10	10	0.22	0.15-0.35	NR
Rainbow Smelt	O	NS	206	50	0.18	0.02-0.20	NR
American Eel	A	NS	4	4	0.16	0.12-0.22	NR
Alewife	H	NS	13	1	0.15	--	NR
Chinook Salmon	S	SS	1	1	0.13	--	9.13
Smallmouth Bass	C	SS	10	10	0.13	0.03-0.27	5.42
Coho Salmon	S	SS	1	1	0.12	--	9.22
Brown Trout	S	SS	12	12	0.11	0.03-0.22	6.54
White Perch	P	SS	4	4	0.11	0.07-0.18	7.51
Brown Bullhead	I	NS	83	83	0.09	ND-0.80	NR
White Perch	P	CB	8	2	0.09	0.05-0.12	1.72
Yellow Perch	PR	NS	80	80	0.09	ND-1.3	NR
White Bass	P	NS	12	12	0.08	0.04-0.13	NR
Northern Pike	E	SS	2	2	0.07	0.04-0.09	2.91
Northern Pike	E	CB	11	4	0.06	0.06-0.08	1.04
American Eel	A	SS	1	1	0.05	--	1.00
Walleye	P	SS	2	2	0.04	0.03-0.05	3.24
Rainbow Trout	S	NS	60	60	0.03	0.005	NR
Carp	CY	NS	4	4	0.02	0.01-0.05	NR
Northern Pike	E	NS	11	11	0.02	ND-0.10	NR
Suckers	CA	NS	50	50	0.02	ND-008	NR
Brown Bullhead	1	CB	10	2	0.02	Trace-0.04	0.57
Black Crappy	C	NS	3	3	0.01	0.008-0.02	NR
Yellow Perch	PR	SS	11	6	0.01	Trace-0.04	1.69
Largemouth Bass	C	NS	3	3	0.004	0.004-0.005	NR
Rock Bass	C	CB	10	1	Trace	--	0.55
Bluegill	C	NS	10	10	ND	--	NR
Chain Pickerel	E	NS	2	2	ND	--	NR
Gizzard Shad	H	NS	3	3	ND	--	NR
Minnows	CY	NS	2	NR	ND	--	NR
Yellow Perch	PR	CB	8	1	ND	--	0.43

Footnotes on next page.

FOOTNOTES FOR TABLE 6.7

[a] Source: New York State Department of Environmental Conservation, (1976).

[b] P	Percichthyidae (temperate basses)
S	Salmonidae (trouts)
I	Ictaluridae (freshwater catfishes)
O	Osmeridae (smelts)
A	Anguillidae (freshwater eels)
H	Clupeidae (herrings)
C	Centrarchidae (sunfishes)
PR	Percidae (perches)
E	Esocidae (pikes)
CA	Catostomidae (suckers)
CY	Cyprinidae (minnows and carps).

[c] NS	Canada/North Shore--Various locations
SS	U.S./South Shore--Various locations
CB	Chaumont Bay.

[d] NR	Not reported

Interestingly, fish with high mirex concentrations have also high PCB concentrations.

Mirex (and Kepone) are monitored in South Atlantic and Gulf Coast states as part of FDA's Compliance Program Evaluation (U.S. Department of Health, Education, and Welfare, 1977). Table 6.8 displays mirex levels in the 132 samples examined. The findings are grouped by State and further by specie group (finfish, shellfish, crustaceans) within each state. It can be seen that none of the surveys of shellfish and crustaceans showed detectable mirex (Alabama, Louisiana, Georgia, including Northeast Florida, Mississippi, and South Carolina). In the case of finfish, however, all surveys except the Texas survey contained some samples with detectable mirex (Alabamam, Arkansas, Louisiana, and Mississippi). The average mirex levels for finfish in the complete surveys within these states were:

- Alabama (0.029 ppm)
- Arkansas (0.021 ppm)
- Louisiana (0.015 ppm)
- Mississippi (0.002 ppm).

Further breakdown of the surveys relative to the geographic area within each state shows some variation in the levels in finfish from different areas within the respective state. For example, in Arkansas the 9 samples from the central area were all negative for mirex and the 2 positive identifications both came from southern Arkansas. This analysis of findings indicates a relationship between mirex-treated areas (1976) and positive survey findings. Shell fish and crustaceans do not appear to support this general observation since all were negative in Southern Louisiana and Mississippi where mirex was applied in 1976.

One factor studied in this survey was the possible co-occurrence of Kepone and mirex in fish resulting from environmental or metabolic conversion of mirex (from mirex bait) to Kepone. This relationship was not observed in this survey since only one sample of crabs from Georgia was found to contain Kepone (trace level) and it contained no detectable mirex. Mirex was found in numerous finfish samples, with no detectable Kepone.

Data on mirex residue accumulation in selected items of the human food chain were also monitored in an extensive study of vertebrates by Collins, et al., (1974). These data are presented in Section 7.

6.5.2 Kepone

The problem of Kepone contamination of fish goes beyond Virginia waters. Kepone has been discovered in fish along the Atlantic Coast from Delaware to North Carolina. In early 1976, FDA established regulatory action levels for unavoidable residues of Kepone in fishery products. These levels, based on recommendations from the Environmental Protection Agency, are as follows:

TABLE 6.8. MIREX FINDINGS BY GEOGRAPHIC AREAS WITHIN SOUTH ATLANTIC AND GULF COAST STATES[a]

		Fin Fish			Shellfish		Crustacea		
State	Location	Number of Samples	Mean ppm Mirex	Survey Mean	Number of Samples	Mean ppm Mirex	Number of Samples	Mean ppm Mirex	
Alabama	Northern	8	.035	.029	.12	N.D.			
	Southern								
	Eastern	4	.018						
Arkansas	Southern	3	.083	.021					
	Central	9	N.D.				1	N.D.	one
Florida	Northern						11	N.D.	survey
Georgia	Eastern								
Louisiana	Northern	1	.022	.015	12	N.D.	12	N.D.	
	Southern								
	Central	11	.014				12	N.D.	
Mississippi	Southern								
	Central	12	.002	.002			12	N.D.	
South Carolina	Eastern								
Texas	Northern	6	N.D.	N.D.					
	Southern	6	N.D.						

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[a] Adapted from U.S. Department of Health, Education and Welfare (1977).

[b] ND = Non detected.

Kepone in oysters, clams and mussels (edible portion)	0.3 ppm
Kepone in finfish (edible portion)	0.1 ppm
Kepone in crabs (edible portion)	0.4 ppm

Currently, these action levels are being enforced, although there is a great deal of agitation by fishermen and other commercially interested parties for an upward revision of these concentrations. Several meetings have been held to discuss this possibility and presently EPA has responded to this request by recommending to FDA that the permissible amount of Kepone in finfish be raised from 0.1 ppm to 0.3 ppm (Anon., 1977).

Kepone has been found in fresh water trout and suckers taken from Spring Creek which runs near the Nease Chemical Plant in State College, Pennsylvania. That plant produced Kepone in 1958-59 and 1963, and produced mirex, which can be converted to Kepone, as late as 1974. Trout contained Kepone in amounts ranging from 0.15 to 0.17 ppm and suckers had Kepone levels as high as 0.18 ppm (Anon., 1976d). Federal standard for human consumption is 0.1 ppm. Pennsylvania officials are presently conducting an extensive survey of fish and waters in the area; however, results are not yet available.

Currently, the FDA Market Basket Program does not monitor foods for Kepone residues, but the FDA is conducting a survey of Kepone residue levels in Chesapeake Bay and East Coast fish. Upon finding traces of Kepone in fish sold in Baltimore, Norfolk, Philadelphia, and New York, and in fish caught off New Jersey and Long Island, FDA announced that it will broaden its monitoring program for fish sold in East Coast cities and for fish, shellfish and crustaceans caught on the East Coast. Unacceptable levels of Kepone were found only in fish sold in Baltimore and Norfolk. (Anon., 1976e).

While the majority of shellfish taken from the James River contain less than the 0.3 ppm action level of Kepone, oyster and clam samples taken from the fall line at Richmond to Hampton Roads contained 0.21 to 0.81 ppm of Kepone. Samples of crabs from Hampton Roads and the Elizabeth River contained 0.45 to 3.44 ppm Kepone. Fish and fish parts samples from the Hopewell, Virginia, area contained from 0.02 to 14.4 ppm of Kepone (Shanholtz, 1976). The observations of high Kepone levels in fish, shellfish, and crabs from the Hopewell, Virginia, area together with the findings of similarly high levels in Baileys Creek (1 to 4 ppb), Appomattox River (greater than 0.1 ppb), James River (0.11 to 0.28 ppb), and 1 to 4 ppm in sediments from the same three locations, prompted a ban on taking fish, shellfish, and crabs from the James River and its tributaries from Richmond to the Chesapeake Bay. The order became effective December 17, 1975 (Shanholtz, 1976).

FDA's findings of excessive levels of mirex and Kepone in Lake Ontario fish caused the State of New York to ban the possession of six species of Lake Ontario fish, including: coho and chinook salmon, brown bullhead, catfish, lake trout, small mouth bass, and alewife herring. Limited consumption of white perch, white bass and smelt was also advised (Whalen, 1976 personal communication; Anon., 1976f). FDA's Buffalo District Office has been working closely with the State of New York in monitoring Lake Ontario fish for mirex and other chemical residues. Because Kepone may be present as an impurity in mirex formulations and mirex can under certain conditions be converted to Kepone, FDA has instructed its district offices located in states where mirex has had extensive use to test commercial fishery products for both mirex and Kepone residues. In view of the proximity to Hooker Chemmical's Niagara Falls plant which produced mirex, FDA's Buffalo District also includes analyses for Kepone residues in Lake Ontario fish samples that are collected under its cooperative program with the State of New York (Wessel, 1976 personal communication).

The data presented below represent recent findings of the FDA Compliance Program Evaluation as of the December 2, 1976, reporting period (Corneliussen, 1976 personal communication). Cumulative Kepone totals are reported in groups according to species and location:

(1) Official Sampling Originating from the Chesapeake Bay

Five additional samples were reported during the November 18 to December 2, 1976, period. This brings the cumulative average level of Kepone to 0.037 ppm for 75 finfish, 0.61 for 11 samples of crabs, and no detected Kepone in 3 samples of oysters and one sample of clams.

The cumulative number of each species examined followed by the average ppm Kepone for that specie are as follows: 21 croaker (0.032 ppm), 21 bluefish (0.046 ppm), 13 trout (0.043 ppm), 8 spot (0.022 ppm), 4 catfish (0.023 ppm), 1 rockfish (none detected), 2 mackerel (0.075 ppm), 3 flounder (0.01 ppm), 2 butterfish (0.05 ppm), 11 crabs (0.061 ppm), 3 oysters (no detectable Kepone), and 1 clam (no detectable Kepone).

(2) Bluefish Caught in the Atlantic Ocean

No additional samples were reported during the past sampling period. Therefore, the total remains at 62 samples under this program. Of this total, Kepone was not detected in 10 Florida samples and 2 North Carolina samples; Kepone levels from traces to 0.03 ppm were found in 42 samples from North Carolina, Delaware, New Jersey, New York, Rhode Island, and Massachusetts; and 8 samples from the Virginia Atlantic Coast contained Kepone in the 0.01 to 0.06 ppm range.

(3) Kepone/Mirex Survey Samples from South Atlantic and Gulf Coast Areas:

> Twenty-four (24) additional samples were reported during the past sampling period, bringing the cumulative sample total under this program to 48 finfish, 28 shrimp, 20 crab samples, and 21 oyster samples from Texas, Louisiana, Georgia, Florida, South Carolina, Mississippi, Arkansas, and Alabama. Of these, only one sample of crabs from Georgia contained a trace of Kepone. Mirex findings from the South Atlantic and Gulf Coast areas are reported in Section 6.5.1.

EPA has recommended to the Food and Drug Administration (FDA) the following "action levels" (allowable temporary levels of pesticide residues to be used as enforcement guides): 0.3 ppm of Kepone in the edible portion of shellfish (oysters and clams), 0.1 ppm in finfish, and 0.4 ppm in crabs. EPA has also recommended a 0.03 ppm action level in processed oyster stew.

An action level is permitted under existing regulations when a food or feed commodity is inadvertently contaminated. Action levels are set on the basis of two primary factors: (1) the level of actual residues found in the affected commodity and (2) the safety of that level to humans based upon probable exposure and extrapolation from toxicological data showing that no greater levels could safely be tolerated in the human diet (Blanchard, 1976).

The action levels considered dietary intake of the commodities carrying the residues. Oysters are only 0.23 percent of man's diet on the average and at the action level of contaminatin would contribute less than one millionth of a gram of Kepone per week. Crabs make up even less, 0.16 percent of man's diet, which is the reason for the higher action level. Finfish make up a larger portion, 0.72 percent, which explains the considerably lower action level recommendation.

Under usual action level procedures, the highest amount of a toxic chemical found not to cause chronic toxic effects in test animals is established; this is then reduced by a factor of 1/100. However, for Kepone, the EPA used a safety factor of at least 1/1,000 because of the extent of public concern with Kepone contamination (Blanchard, 1976).

Control of the banana root borer constitutes the only food or feed use of Kepone. Although the Kepone is applied to the ground and not to the plant, there is some possibility of contamination of the fruit. Originally, EPA regulations allowed a tolarance of 0.01 ppm of Kepone in or on the fruit (40 CFR 180.287). This tolerance for bananas was considered safe on the basis of essentially no residue in the edible pulp, rather than on supportive toxicological data (U.S. Environmental Protection Agency, 1975a). Residues up to the level of the established tolerance were presumed to be present on the skins but residues, if any, in the edible pulp of the bananas were presumed, on the basis of the evidence submitted with the registration petition, to be less than the sensitivity of the analytical method (GC), estimated at 0.005 ppm. This tolerance was revoked, effective January 27, 1978 (U.S. Environmental Protection Agency, 1978).

Kepone has also been used to control wireworms in tobacco fields. The persistence of Kepone in soils suggests the possibility of carry over of Kepone residues to food crops grown in rotation with Kepone treated tobacco. EPA reports that no studies addressing this topic have been located (U.S. Environmental Protection Agency, 1975a).

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7.0 ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

7.1 SUMMARY

The principal route of entry of mirex into the environment has been the aerial application of mirex bait, applied to millions of hectares in the southeastern United States for fire ant control. Residues of mirex in the U.S. environment are at low levels, frequently approaching the limit of detection. This is consistent with the rate of application which has been utilized for fire ant control, normally 4.2 g/ha (1.7 g/ac), and the total cumulative application of probably less than 250,000 kg (550,000 lb) during the 1962 - 1975 period (see Section 2.5.1.2). Theoretical concentration in the top 7.5 cm (3 in) of soil at recommended rates of application would, assuming no removal by fire ants, be 4 to 5 ppb; experimental soil analyses bracket this value, most are below this.

Less generally recognized is the potential contribution to environmental contamination of mirex ($C_{10}Cl_{12}$) marketed as a fire retardant additive for polymeric materials under the name Dechlorane. The contamination in Lake Ontario is partially from wastes from mirex-Dechlorane manufacture, and partially from a Dechlorane spill, and is not from any insecticidal application of mirex. Sediment analyses indicate that several hundred kilograms of Dechlorane (mirex) are trapped in lake sediments nearest the two discharge points.

The measured solubility of mirex in water is 1 ppb or below, and mirex in natural waters is frequently undetectable at a 0.01 ppb sensitivity. Experimentally, water concentrations of 1 to 10 ppt have been detected in regions treated for fire ant control. Concentrations in sediments are higher, typically by a factor of 10 to 20.

All evidence points toward a high degree of stability for mirex in the environment, whether in soil or sediment. It has been suggested that the half-life may be 12 years (or more); this is supported by experimental field determinations of mirex in soil and sediment which had been exposed for such a period. Kepone, incidentally, appears to be a major intermediate degradation product of mirex.

Kepone has no significant agricultural uses in the U.S. The principal route of entry of Kepone to the U.S. environment, unlike mirex, has not been the result of its insecticidal application but the result of waste discharges and losses during its manufacture at Hopewell, Virginia. The amount of Kepone which has escaped from its principal U.S. use in ant and roach traps appears to be virtually undetectable.

Prior to the Life Science Products Company incident, essentially no data existed on environmental concentrations of Kepone. In connection with the investigation of that incident, hundreds of analyses have been performed on air, water, soils, sediments, and biota from the area in and around Hopewell, and from the James River and Chesapeake Bay. Contamination of James River sediments by Kepone from Hopewell nearly to Newport News has been demonstrated. It has been estimated that as much as 45,000 kg of Kepone may lie on the bottom of the James River. The mobility of this Kepone is anticipated to depend on the mobility of the sediments. Since Kepone, like mirex, is resistant to biodegradation, this inventory will pose a problem for many years.

Field studies have shown that mirex tends to bioconcentrate in some species, especially in predaceous species. Although the residue levels found generally appear to be below those causing acute toxicity, the long-range effects of low chronic dosages are conjectural.

7.2 DISTRIBUTION OF MIREX IN THE PHYSICAL ENVIRONMENT

7.2.1 Entry Into the Environment

The principal route for the entry of mirex into the environment has been the aerial application of mirex bait, used in the southeastern states for fire ant control. Areas treated and annual quantities are shown by Table 2.4. As noted in Section 2.5.1.2, the total quantity of mirex applied between 1962 and 1975 may have approached 250,000 kg (550,000 lb). Theoretically, all of this was applied to land. However, the precision of aerial application with the fixed wing planes employed was not such that spill over to aquatic areas can be ruled out. It seems quite likely that some mirex was inadvertently applied over water. In recent years helicopters and small aircraft have been used; precision of application appears to be good with these methods.

The role of Dechlorane in the contamination of the environment by $C_{10}Cl_{12}$ (mirex) should not be overlooked. Most of these identical chemical compounds produced between 1959 and 1967 were manufactured at the Hooker Chemical plant at Niagara Falls, New York. Aqueous wastes from this plant were discharged either into the Niagara River or into the Niagara Falls sanitary waste system, whose effluent was also discharged into the Niagara River. The Niagara River empties into Lake Ontario, in which $C_{10}Cl_{12}$ (Dechlorane-mirex) contamination has been found, as described in a later section. There appear to be no data on the quantities of such discharges during this period.

All Dechlorane-mirex has been ground and packaged at the Hooker Niagara Falls plant both during the period when it was manufactured there and subsequently. Material is ground for approximately one week per year and the grinder is washed after the week-long campaign. An analysis of this cleanup operation in 1974 indicated that 136 liters (36 gallons) of aqueous waste, containing 62 ppm of $C_{10}Cl_{12}$, was discharged to the Niagara Falls sanitary sewer system (Chambers, 1976).

Results of another survey, by EPA Region II personnel in January 1975, indicated a level of 270 ppm of mirex in 125 gallons of cleanup water resulting from the reprocessing of 1,360 kg (3,000 lb) of mirex. A Region II field inspector estimated in his report of the visit that, at a minimum, Hooker discharges 9 kg (20 lb) of mirex per year to the Niagara Falls Sewage Treatment Plant (STP) (Legro, 1976).

Automated dust collectors, rated at 99.9 percent collection efficiency by the manufacturer, are used for the grinding operation. No visible discharges have been observed by Company or government personnel (Chambers, 1976).

Subsequent to 1967, when manufacture of mirex-Dechlorane by Hooker was terminated, the Nease Chemical Company at State College, Pennsylvania, supplied the product, from 1968 to 1969 and again from 1973 to 74 (see Section 2.5.1.1). No data on losses from the Nease Chemical Company operations have been found.

Another possible source of entry of mirex-Dechlorane into the environment is associated with the manufacture, use, and disposal of products containing Dechlorane fire retardant additive. Unlike mirex, essentially all of which went to the southeastern U.S., Dechlorane was distributed to customers for a wide variety of uses (plastics, pyrotechnics, roofing, and elastomers) at a multitude of North American locations, including Canada. The ultimate fate of these products, which represented three times as much $C_{10}Cl_{12}$ as was used for fire ant bait, is uncertain.

7.2.2 Soil

At the standard application rate of 1.4 kg/ha (1.25 lb/ac) of 4X bait, approximately 4.2 g/ha (1.7 g/ac) of active ingredient was applied. It has been calculated that this is equivalent to approximately 4 ppb (Mirex Advisory Committee, 1972[*]) or 5 ppb (Markin, et al., 1972) in a standard 3-inch soil sample. Mirex concentrations in soil have been found to be of this order of magnitude. The two laboratories most active in monitoring efforts have been the USDA Gulfport, Mississippi, laboratories, and the EPA laboratories at Gulf Breeze, Florida, along with various state agencies in the southeastern U.S. Unfortunately, results of these studies have, in general, not been published (Alley, 1973).

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[*] At the reduced 0.45 g/ac application rate, initiated in 1977, the corresponding average soil concentration is only 1 ppb.

Mirex residues in soils from open pasture following normal aerial treatments have generally ranged from 0.1 to 10 ppb. (Mirex Advisory Committee, 1972). Markin, et al., (1972) reported that in two studies, the residues in soils in open pastures, away from ant mounds, ranged from 0.7 to 2.5 ppb. The highest amount of mirex detected in soil was 100 ppb, found in the soil of a recently treated ant mound and probably represented mirex concentrated in the mound by ants.

Spence and Markin (1974) reported on the time-variant soil concentrations for two field test plots in Louisiana and Mississippi. Each site also contained one or more ponds so that water and sediment concentrations were also available. Soil mirex concentrations (7.5 cm depth) ranged from 0.2 to 2.5 ppb at the Louisiana site, and from 0.3 to 10.4 at the Mississippi site, as illustrated in Table 7.1. At the 4.2 g/ha (1.7 g/ac) rate of application, the expected residue level in the upper 7.5 cm of soil would be about 4 ppb. In the Louisiana plot the concentrations found immediately after treatment were much lower, possibly indicating collection and removal by foraging fire ants. The gradual subsequent buildup was suggested by the authors as a possible result of excretion or decomposition of dead insects. The much greater variability in Mississippi results was believed to be possibly due to the winter (February) application of mirex, when insect activity was minimal; Louisiana application had been made in May.

Johnson, et al., (1976a,b) and Bevenue, et al., (1975) described the results of the 1972-1974 monitoring program on the treatment of pineapple fields with mirex. 4X bait was applied at the rate of 6.2 kg/ha (2.5 lb/ac), i.e., 8.4 g/ha (3.4 g/ac) of active ingredient, which is twice the standard rate for fire ant control. Results of the analysis of eight soil samples taken at varying times up to 9 months after the mirex application are tabulated below. The first sample in each field represented background levels 1 week prior to treatment.

Field 233		Field 234	
Date	Mirex, ppb	Date	Mirex, ppb
October 6, 1973	ND	October 6, 1973	ND
November 3, 1973	3	November 2, 1973	9
January 19, 1974	4	January 19, 1974	5
April 21, 1974	ND	April 21, 1974	9
July 13, 1974	ND	July 13, 1974	9

ND = Not detected.

7.2.2.1 Mobility and Persistence in Soil--

Several bits of evidence point towards a long persistence of mirex in soil. It is essentially insoluble, so that dissolution does not appear likely to be a major mechanism for translocation. Laboratory experiments indicated that decomposition by photolysis proceeds very slowly. As the

TABLE 7.1 MIREX RESIDUES IN SOIL COLLECTED IN MISSISSIPPI (1972) AND LOUISIANA (1971-72)[a]

Days Posttreatment	Mirex, ppb
Mississippi	
Pretreatment	—
1-3 hours	6.3
1	0.71
2	0.31
3	4.2
7	0.87
14	0.41
21	0.22
28	1.2
35	1.3
42	4.0
49	0.78
56	2.7
63	8.2
70	1.5
77	1.2
84	2.0
91	10.4
98	2.4
105	1.1
112	0.31
Louisiana	
Pretreatment	—
1-3 hours	0.70
3	0.70
4	0.30
7	0.80
17	0.70
25	2.0
31	2.0
38	2.5
45	2.5
54	2.5
69	1.4
77	1.4
90	1.0
117	1.0
164	0.60
185	0.90
223	0.90
268	0.20
337	0.20
374	—

[a] Source: Spence and Markin (1974).

[b] — = No data

Council for Agricultural Science and Technology (1976) noted, mirex is extremely resistant to biological degradation.

It has been estimated that mirex has a half-life of 12 years (Holden, 1976). This may actually be an underestimation, judging by the findings of Carlson, et al., (1976) who were able to experimentally account for about 50 percent of originally applied mirex after a 12-year period. However, their data do show a number of degradation products, including Kepone (a major degradation product) and dechlorinated mono and dihydro products demonstrative of a pathway by which mirex can ultimately disappear from the environment.

Physical runoff is a possible transfer mechanism between the soil and water compartments. Actual runoff experiments on mirex do not appear to have been reported. Water and sediment analyses have been conducted, but direct aerial deposition in the water body or stream appears to have also been a possibility, so that runoff could not be measured quantitatively.

Pesticide runoff from a Mississippi Delta watershed has been experimentally determined by Willis, et al., (1976), who showed mean annual concentrations of trifluralin, DDE, DDT, and toxaphene of 0.2 to 11 ppm in field runoff. The low solubility of mirex would probably preclude the attainment of such concentrations, but mirex could easily be associated with a mean sediment loss such as the 27.6 metric tons/ha (12.3 tons/ac) also observed in this study.

7.2.3 Water and Sediments

As noted in Section 2.3.1.1, the theoretical solubility of mirex is in the ppb range or below. Water has evidently proven to be a difficult substrate to work with, and it has been difficult to differentiate between mirex in solution and that associated with ultra fine suspended material present. Markin, et al., (1972) reported that earlier analyses of 50 water samples collected routinely following mirex application failed to detect mirex with techniques possessing 0.01 ppb sensitivity. Borthwick, et al., (1973) reported a similar lack of success in detecting mirex in estuarine samples. Alley (1973) also states that mirex has not been observed in natural waters with analytical techniques sensitive to 0.01 ppb. Some of the work at his laboratory on water from a creek whose entire watershed had been treated with mirex bait indicated that a substance tentatively identified as mirex can be observed at the 0.1 ppt level, an almost imperceptible level, and one which the authors suggest may have an insignificant environmental impact. The Mirex Advisory Committee (1972) reported that several investigators had found mirex residues of 0.5 to 1.0 ppb in pond water samples, but these were from ponds pretreated with 0.5 to 1.0 ppm mirex, respectively.

Spence and Markin (1974) also analyzed time-variant mirex concentrations in pond water and sediments in two Mississippi and Louisiana mirex-treated test plots analyzed for soil concentrations.

Water was sampled by passing it successively through a cellulose cartridge filter (sediment) and an activated charcoal cartridge filter (dissolved substances). Samples were taken at the surface and at the bottom (1.5 m depth). Sediments were sampled at 10 locations around the pond.

Results of the water analyses (Mississippi test plot) are shown in Table 7.2. Water samples from the bottom of the pond showed residue values which remained higher and more constant than those from the surface of the pond. Most of the results, at either depth, were in the parts per trillion range. Results of the sediment analyses are shown in Table 7.3. As in the soil samples, the results for Mississippi were higher and more variable, but all samples (with one exception) were below 1 ppb.

In view of the insolubility of mirex in water and the rapid adsorption onto particulate matter which ultimately settles to the bottom, mirex is not expected to be subject to photochemical degradation in estuarine environments. Because mirex resists decomposition and is virtually inert under normal environmemntal conditions, it will not remain long in the water column but may have an appreciable lifetime in the sediments. (See Sections 2.3.2.1 and 7.2.3.1). Transportation of bait particles by surface runoff is a more likely source of stream and lake contamination (Jones and Hodges, 1974).

The Mirex Advisory Committee (1972) reported sediment sample concentrations of 0.01 to 21 ppb from fresh water ponds pretreated with mirex; Van Valin and O'Donnell (1968) found ranges of from 0.09 to 32.7 in sediments from four ponds treated with 0.1 ppm of mirex.

In the work of Borthwick, et al., (1973) on the behavior of mirex in estuaries, analysis of sediment samples for mirex gave negative results (0.01 ppm) except in six instances, in which concentrations ranged from 0.01 to 0.07 ppm.

A number of field studies indicate that mirex is very stable in mud and sediments. The analyses reported by Carlson, et al., (1976) of muck samples from a pond bottom at the site of a crash of a plane carrying mirex bait showed a concentration of 0.27 ppm of mirex plus degradation products. This is of course, a special case, and is not representative of soil concentrations to be expected from the utilization of mirex for fire ant control.

Further evidence of the stability of mirex in sediments is furnished by recent data from Lake Ontario. Mirex was first reported in fish caught in northern Lake Ontario by Kaiser (1974). Following this discovery, several hundred sediment samples recovered by a large scale geological survey of the lake conducted by the Canadian Centre for Inland Waters in 1968 were analyzed for mirex.

TABLE 7.2 MIREX RESIDUES IN FILTERED WATER COLLECTED IN MISSISSIPPI (1972).

	Mirex Concentration, ppb					
	Filter System 1 (Deepest Point)			Filter System 2 (Pond Surface)		
Days Posttreatment	Cellulose Cartridge	Activated Carbon Cartridge	Total	Cellulose Cartridge	Activated Carbon Cartridge	Total
Pretreatment	Neg	Neg	Neg	Neg	Neg	Neg
1-3 hours	0.033	Neg	0.033	0.007	0.009	0.016
1	0.530	Neg	0.530	0.008	0.012	0.020
2	0.001	Neg	0.001	0.003	0.004	0.007
3	0.001	0.006	0.007	0.001	0.002	0.003
7	0.001	0.007	0.008	0.005	0.001	0.006
14	0.003	0.010	0.013	0.006	0.001	0.007
21	0.002	0.012	0.014	0.002	0.003	0.005
28	Neg	0.010	0.010	0.001	0.002	0.003
35	0.001	0.012	0.012	Neg	0.002	0.002
42	Neg	0.003	0.003	Neg	0.001	0.001
49	0.004	0.004	0.008	Neg	0.002	0.002
56	0.002	0.003	0.005	Neg	Neg	Neg
63	0.001	0.009	0.010	0.001	0.001	0.002
70	0.001	0.010	0.011	0.001	0.001	0.001
77	0.001	0.005	0.006	0.001	0.001	0.001
84	0.001	0.010	0.011	0.002	Neg	0.002
91	0.002	0.003	0.005	0.002	0.001	0.003
98	0.001	0.015	0.016	0.003	Neg	0.003
105	0.001	0.003	0.004	0.007	0.003	0.010
112	0.001	0.002	0.003	0.005	0.002	0.007

[a] Source: Spence and Markin (1974).

[b] Neg = No mirex at level of detection.

TABLE 7.3 MIREX RESIDUES IN SEDIMENT COLLECTED IN MISSISSIPPI (1972) AND LOUISIANA (1971-72) [a]

Posttreatment	Mirex, ppb
	Mississippi
Pretreatment	0.06
1-3 hours	0.16
1	0.41
2	0.17
3	0.51
7	0.11
14	0.11
21	0.30
28	0.93
35	1.1
42	0.85
49	0.04
56	0.03
63	0.20
70	0.01
77	0.02
84	0.05
91	0.10
98	0.01
105	0.76
	Louisiana [b]
Pretreatment	Neg
1-3 hours	Neg
3	Neg
4	Neg
7	0.02
17	0.04
25	0.30
31	0.70
38	0.60
45	0.03
54	0.08
69	0.10
77	0.10
90	0.60
117	0.30
164	0.20
185	0.09
223	0.06
268	0.50
337	0.16
374	0.40

[a] Source: Spence and Markin (1974).

[b] Louisiana rainfall not recorded.

[c] Neg = No mirex at level of detection.

Analysis of these sediment samples revealed the occurrence of mirex in two anomalous zones which coincided with input from the Niagara River in the western end of the lake, and from the Oswego River at the eastern end. The occurrence of these anomalies was confirmed by a 1976 resampling program conducted jointly by Canada and the New York State Department of Conservation. The Hooker plant at Niagara Falls is the presumed source of the Niagara River "hot-spot". As a result of the analysis of bottom sediment samples taken up the Oswego River, the Armstrong Cork Company manufacturing plant located 14 km upstream of the river mouth was identified as an industrial source. Known use of Dechlorane fire retardant by this plant indicated a substantial loss (estimated at 450 kg) approximately 15 years ago. Mirex concentrations of sediments in the vicinity of this plant ranged from 100 to 1600 ppb (Thomas, et al., 1978).

Of the 229 samples analyzed, only 75 (33 percent) contained detectable residues of mirex. Concentrations of mirex in the 75 samples ranged from 0.8 to 40 ppb, with a mean of 6.9 ppb and a standard deviation of 8.3 ppb (Thomas, et al., 1978). The distribution of mirex is shown in Figure 7.1. No mirex was detected over a large portion of the lake, particularly in the northern half. The two high concentration zones are quite evident. Thomas, et al., made estimates of the possible mirex inventory in the top 3 cm of sediment, based on sediment analyses. These calculations, summarized in Table 7.4, suggest that several hundred kilograms of mirex may be trapped in Lake Ontario sediments.

A water quality criteria value for mirex of 0.001 ppb is suggested by the recently issued Quality Criteria for Water (U.S. Environmental Protection Agency, 1976a). This is based on applying a 1/100 safety factor to the lowest levels at which effects on crayfish have been observed. This level may be difficult to apply and interpret, since it lies at the lower detection limit for mirex.

7.2.3.1 Mobility and Persistence in Water and Sediments--

There are few data bearing on the mobility and persistence of mirex in water and in sediments. The solubility in water is so low that mirex concentrations appear more often than not to be below the limit of detection, precluding the kind of mass balances needed to assess transport. The water analyses available suggest that water is not a significant transport mode.

Mirex in sediments appears to be subject to decomposition mechanisms similar to those in soil, except for the absence of exposure to photolysis. Accordingly, persistence can be expected to be high. The investigation of Carlson, et al., (1976) on the Sebring, Florida, muck (which had received a massive dose of mirex as a result of a plane crash) showed the presence of decomposition products analogous to those found in aged soil samples from another site. The presence of mirex in sediments in the Oswego River (Section 7.2.3) presumably 15 years after its original discharge is further evidence of its stability.

On the basis of the earlier work by Hill and McCarty (1967) on chlorinated hydrocarbons and the more recent work on mirex by Andrade and

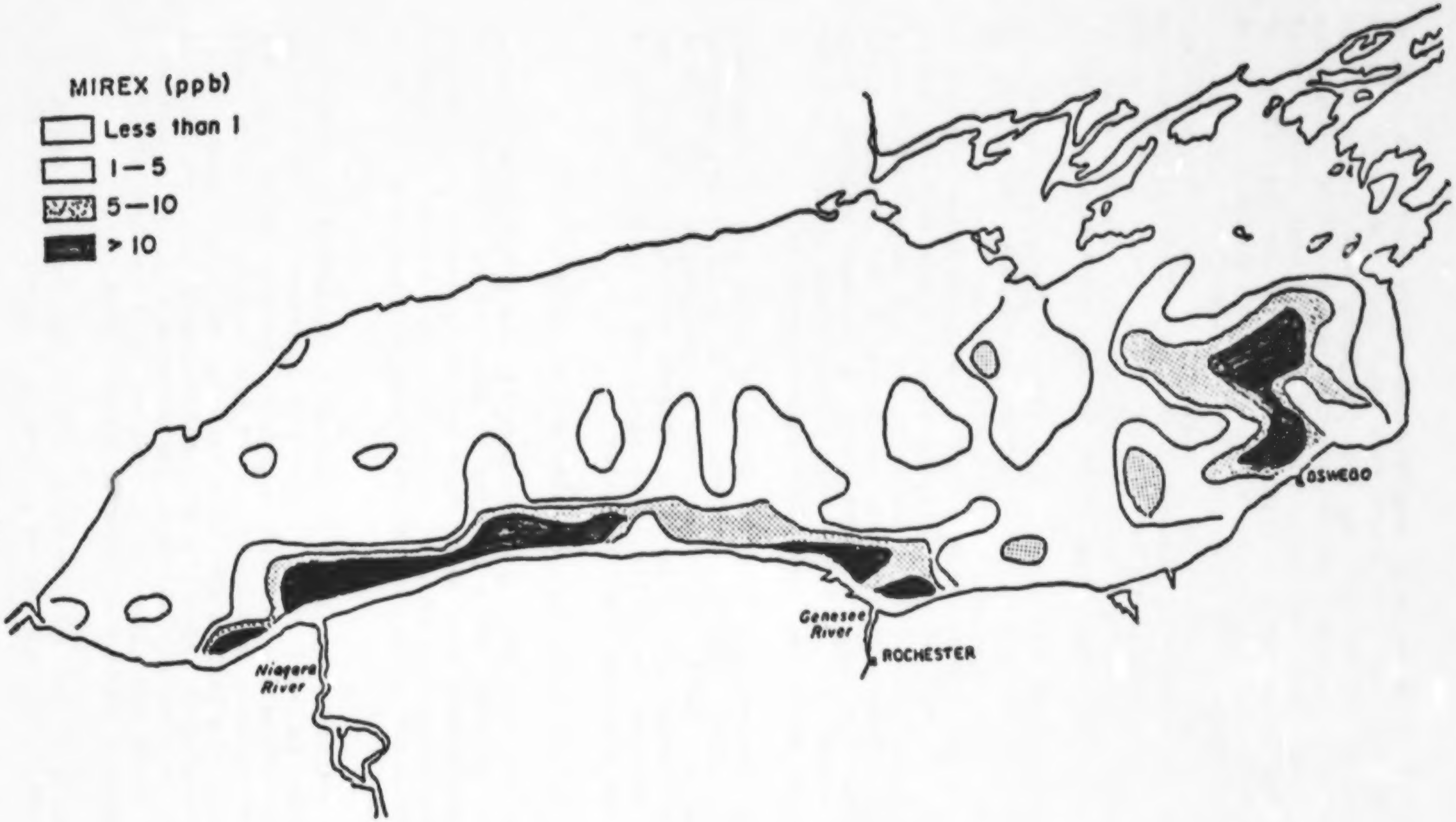
MIREX (ppb)
Less than 1
1—5
5—10
> 10
OSWEGO
Genesee River
ROCHESTER
Niagara River

Figure 7.1. Mirex concentration in Lake Ontario sediments

Source: Thomas, et al (1978). Reprinted, with permission, from J. Great Lakes Research. (c) Internat. Association for Great Lakes Research (1978).

TABLE 7.4. MIREX IN LAKE ONTARIO SEDIMENTS - 1968[a]

Occurrence	Lake Surface Area, km^2	No. of Samples	Mirex in Dried Sediment, ppb		Mirex in 3 cm Depth,[b,c] kg
			Mean	Standard Deviation	
Niagara Anomaly	2349	30	10.0	11.0	366
Oswego Anomaly	1967	27	7.3	6.7	224
Other Samples	1117	16	5.6	9.3	98

[a] Source: Thomas, et al (1978). Reprinted, with permission, from J. Great Lakes Research. (c) Internat. Association for Great Lakes Research (1978).

[b] Sediment density of 2.6.

[c] 80 percent assumed mean water content (mean value for surficial 3 cms from 11 cores).

Wheeler (1974), anaerobic decomposition is a possibility. More research appears to be needed in this area.

7.2.4 Air

No analyses of concentrations of mirex in the atmosphere have been found in the literature. Since mirex in use is dispersed through the air only in association with relatively coarse corn cob grit particles which would settle out rapidly, amounts remaining airborne would be expected to be insignificant. With its high melting point (485 C) and very low vapor pressure (6×10^{-6} mm Hg at 50 C), volatilization from bait on the ground would also be expected to be insignificant.

7.3 DISTRIBUTION OF KEPONE IN THE PHYSICAL ENVIRONMENT

7.3.1 Entry Into the Environment

Kepone's principal use has been in tropical regions outside of the United States for control of the banana root borer. The principal uses in the U.S. have been for control of ants and roaches around homes and buildings and for wireworm control in the southeastern states, but these have been insignificant, relatively consuming only between 1 and 10 percent of U.S. production. As summarized in Section 2.5.2.2, this might represent a total usage of no more than 15,000 to 150,000 kg of Kepone since its commercialization in the late 1950's. It is clear that Kepone was a very minor insecticide. As noted in EPA's Position Document 3, because of its small domestic use, which was primarily nonagricultural, Kepone was given low priority on the list of pesticides for which information on residues was sought and on which research was conducted (U.S. Environmental Protection Agency, 1976b).

Some early registered uses of Kepone were allowed only if no residues could be detected. However, as methods of residue detection became more sophisticated, the expectation of finding no residues of any pesticide used on food crops became unrealistic. In June, 1965, a committee of the National Academy of Sciences, National Research Council, evaluated the practice of registering pesticides for use on food crops on such a basis and concluded that it should no longer be allowed (U..S. Environmental Protection Agency, 1976a).

On February 1, 1968, all uses of Kepone on food crops were cancelled except for bananas. A tolerance of 0.1 ppm in whole bananas was established on November 4, 1970.* Subsequently, registration was granted for use of a 5 percent Kepone dust formulation against banana-root borers (U.S. Environmental Protection Agency, 1976a).

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*As noted in Section 6.3.2, this tolerance was revoked on January 27, 1978.

Most of the Kepone used in the U.S. is contained in traps or dispersed indoors or near homes. Thus, in normal use, there would be very little reason to determine soil, water, and air concentrations of Kepone, and, not unexpectedly, such data are not to be found in existing literature.

Had it not been for the Life Sciences Products Company incident at Hopewell, Virginia, data on environmental concentrations of Kepone would still be essentially non-existent. However, as a result of that incident, hundreds of Kepone analyses have been performed during the past two years on soil, water, and air samples from the area in and around Hopewell, and from the James River and Chesapeake Bay. Sampling and analysis is still continuing, and it will be some time before formal reports on this incident appear in the scientific literature. Preliminary reports and memoranda have been prepared by the various State and Federal agencies involved in the investigation, and the following discussions are based on these.

It should be recognized that these environmental concentrations are in no way representative of the intended use of the product, but are, instead, the result of massive unapproved Kepone discharges from a single point source and represent a unique situation, having no counterpart elsewhere in the U.S.

There is considerable uncertainty as to how much Kepone has escaped into the environment during its manufacture at Hopewell, Virginia. Some solubilized Kepone was lost routinely from the precipitation and washing of the Kepone product, and EPA estimates of these quantities have been compiled (Smith, 1976; U.S. Environmental Protection Agency, 1976c). These were small quantities, less than 1 kilogram from Allied's 1971-74 semiworks operations, and perhaps 25 kg from LSPC operations in 1974-75. Compared with the 45,000 kg (100,000 lb) which the Virginia Institute of Marine Science has estimated lies on the bottom of the James River (U.S. Environmental Protection Agency, 1976d), these quantities are trivial. Although the records of operations at LSPC were fragmentary and incomplete, subsequent hearings have indicated the environmental contamination of the James River resulted from unapproved discharges of gross quantities of Kepone from this plant. The actual amount will probably never be known, but it appears certain that it was thousands of kilograms.

7.3.2 Soil

Dust collection was very poorly handled at LSPC, and the local neighborhood was subjected to considerable fallout. Local air concentrations were very high (see Section 7.3.4), diminishing with distance from the plant.

Soil samples taken adjacent to the LSPC manufacturing site on Terminal Street contained from 1 to 2 percent Kepone (10,000 to 20,000 ppm). Surface soil samples taken due east of the manufacturing site also contained up to 300 ppm of Kepone with the more distant surface soil samples -- up to approximately 1000 meters (3000 feet) from the manufacturing site -- containing 2 to 6 ppm of Kepone (U.S. Environmental Protection Agency, 1975a). It has been estimated that as much as 1 metric ton may have been deposited on the ground within a 1000-meter (3000-foot) radius of the plant (Blanchard, 1976). No data on soil concentrations beyond the Hopewell corporate limits were found.

7.3.2.1 Mobility and Persistence in Soil--

Gawaad, et al., (1971) investigated the leaching of Kepone and other organochlorine and organophosphate insecticides from several types of Egyptian soils. Simple percolation tests were conducted, using soil-filled metal cylinders perforated at the bottom to permit collection of leachate. The insecticides and their degradation products were leached more readily from light soils such as sandy loams and calcareous sandy loams than from heavier soils like clay loam and clay. The amount of applied pesticide which was recovered in the leachate from a 24-hour period ranged from 1.2 percent in an El-Mansoura clay loam to 36.8 percent in a Sacka sandy clay loam. In all cases, the chlorinated hydrocarbons were leached more readily than the organophosphates (Gawaad, et al., 1971). According to a U.S. EPA report (1975b), Gawaad, et al., concluded that contamination of lake or river water could result from the use of Kepone.

Although little is known about the persistence of Kepone in soil, data submitted by Allied Chemical Corporation showed essentially no decline in residual levels 154 days after treatment (U.S. Environmental Protection Agency, 1975b). Clearly, Kepone is very stable and persistent in soils. The EPA report expressed two major concerns regarding the effects of Kepone on soils. First, because oxidation of nitrite to nitrate is depressed after Kepone application, a temporary buildup of nitrite may occur in treated soil, which in turn, may adversely affect some plant species. Secondly, the persistence of Kepone in soils suggests the possibility that residues may be taken up into edible portions of rotational crops such as soybeans and small grains, although no experimental data were available on this topic.

There are very few data available on the persistence of Kepone in soil. By analogy, it would be expected to exhibit almost as much stability as mirex, and to have a long half-life in soil.

7.3.3 Water and Sediments

The preliminary 1975 survey results (U.S. Environmental Protection Agency, 1975a) indicated that in the Appomattox River upstream of the plant, Kepone was detected at 0.1 ppb; in the James River near the mouth of Bailey's Creek levels up to 0.3 ppb were measured. Kepone concentrations in other samples from the James River ranged from 0.1 to 4 ppb. Kepone was undetectable (50 ppt) in the York River, the next river basin north of the James River, which also empties into Chesapeake Bay above the James River.

An ice processing plant, which crushed and bagged ice made elsewhere, was located across the street from the LSPC plant about 50 meters (150 feet) away. The 1975 survey indicated that ice stored at this facility contained from 0.1 to 1 ppm of Kepone. Subsequent samples taken in December, 1975, 4 months after closure of the LSPC plant, were found to contain 1 to 50 ppb. The processor was closed and moved to another location for approximately 8 months (Virginia Department of Agriculture and Commerce, 1977).

Tap water from the Hopewell area was determined to contain no detectable Kepone in the preliminary 1975 survey (U.S. Environmental Protection Agency, 1975a); this was confirmed by a December, 1975, sample (Virginia Department of Agriculture and Commerce, 1977).

River bottom sediment analyses showed nondetectable levels of Kepone (10 ppb) in the western reaches of the Appomattox River, upstream of the Kepone manufacturing site. In the area of Bailey's Creek, into which the Hopewell STP discharges, bottom sediments ranged between 1 and 4 ppm. Sludge samples from the STP holding pond and sludge disposal landfill contained from 200 to 600 ppm Kepone (U.S. Environmental Protection Agency, 1975a). Later sampling (January - May, 1976) by the Virginia Water Control Board, found levels in sediments in Bailey's Creek as high as 30 ppm. Bailey Bay sediments had Kepone concentrations of 1 to 10 ppm in three-fourths of the samples. Down river of this, sediment concentrations were somewhat erratic, varying between 0.02 and 1 ppm (U.S. Environmental Protection Agency, 1976d). The high levels in the STP and in Bailey's Creek appear to be the result of the discard of quantities of Kepone from the manufacturing plant through the Hopewell sewer system, and not runoff from Kepone deposited on the soil in the vicinity of the plant.

In 1976, the U.S. Army Corps of Engineers undertook several series of water and sediment tests to determine the extent of Kepone contamination in the James River navigation channel and the effects of channel dredging on redispersion of the Kepone. The first series of these tests was performed during January, February, and March, 1976, in order to determine whether Kepone was present at various locations on the James River including the Hampton Roads and Elizabeth River area. The results of these tests (Table 7.5) indicated that a Kepone contamination problem existed from Hopewell, Virginia, nearly to Newport News, Virginia (U.S. Army Corps of Engineers, 1976). Nine sediment samples taken in the Hampton Roads area of Newport News showed undetectable Kepone (0.1 ppb), indicating that Kepone redispersion would not be anticipated to result from dredging projects in this area, which is about 110 km (69 mi) downstream from Hopewell.

TABLE 7.5 SUMMARIZED SEDIMENT AND WATER ANALYSES, JANUARY, 1976 [a]

Location	Approximate River Mile	Kepone Concentration	
		Sediment, ppm	Water, ppb
Allied Pier	69	0.46	<0.05
Windmill Point	57	0.41	<0.05
		0.81	<0.05
		0.87	
Dancing Point	42	0.48	<0.05
		0.40	
Goose Hill Shoal	27	0.30	0.04
Skiffes Creek	20	0.09	<0.05

[a] Source: U. S. Army Corps of Engineers (1976).

TABLE 7.6 RESULTS OF ANALYSIS OF THREE CORES FROM WINDMILL POINT SHOAL TAKEN BEFORE DREDGING.[a,b]

Core Depth		Kepone, ppm		
		Core Location		
cm	In	L. Windmill Point Upstream end	L. Windmill Point Middle	L. Windmill Point Lower End
0-15.24	0-6	0.23	0.27	0.09
15.24-30.48	6-12	0.39	0.35	0.07
30.48-45.72	12-18	0.24	0.14	0.04
45.72-60.96	18-24	0.14	0.34	0.05
60.96-76.20	24-30	—(c)	-- (c)	0.02

[a] Source: U. S. Army Corps of Engineers (1976).

[b] Samples taken in January, 1976.

[c] Not samples at this depth.

In another Corps of Engineers test, sediment cores from the Windmill Point area just below Hopewell (River Mile 57) were analyzed to a depth of 76 cm (30 in). These results, shown in Table 7.6, indicate that the near-field sediments are contaminated with Kepone to a considerable depth.

Profiles of James River sediment Kepone concentrations have been developed by the U.S. Environmental Protection Agency, as of mid-1976, from Hopewell to Newport News, as shown by Figures 7.2, 7.3, and 7.4 (U.S. Environmental Protection Agency, 1976d). Maximum concentrations were found at Bailey's Creek (∼10 ppm), with the next highest concentrations being 1-10 ppm in Bailey Bay. Beyond this point, diffusion and dispersion and sedimentation patterns control the distribution of the Kepone. A second series of Corps tests, during June, July, and August, 1976, conducted as part of a "test dredging" near Windmill Point on the James River were undertaken in conjunction with live box biological tests by the Virginia State Water Control Board. The purpose of this "test dredging" was to determine the effect, if any, that maintenance dredging as currently conducted on the James River would have on the Kepone situation. No special measures were taken during the "test dredging" to reduce the effects. The results of the Corps tests indicated that even though Kepone was released near the dredge pipe outfall in the dredged material disposal area, the concentration of Kepone in the water column at two locations 500 feet away from the outfall was near pre- and postdredging levels (U.S. Army Corps of Engineers, 1976). Analyses of water samples taken just before these dredging tests showed Kepone concentrations of 0.07 to 0.13 ppb in unfiltered samples and 0.05 to 0.12 ppb in filtered (0.45 micron filters) samples.

Work is continuing on this problem. Attempts are currently under way at the EPA Environmental Research Laboratory at Gulf Breeze, Florida (ERL/GB), the Virginia Institute of Marine Sciences (VIMS), and the Waterways Experiment Station (WES) of the U.S. Army Corps of Engineers at Vicksburg, Mississippi, to develop an estuarine model that will predict the fate and transport of Kepone in the aquatic environment (Blanchard, 1976).

7.3.3.1 Mobility and Persistence in Water and Sediments--

Very few data are available on this topic. Kepone's mobility in sediments will depend on the mobility of the sediments themselves. In the James River, where sediment movement is substantial, at least down to Newport News, the Kepone appears to have been transported along with the sediments. However, the conclusion is that the Kepone-carrying James River sediments will be essentially trapped in the lower estuary, i.e., from generally Jamestown Island to the Chesapeake Bay, where the river becomes broad and shallow, and where tidal inflow becomes operative (U.S. Environmental Protection Agency, 1976d).

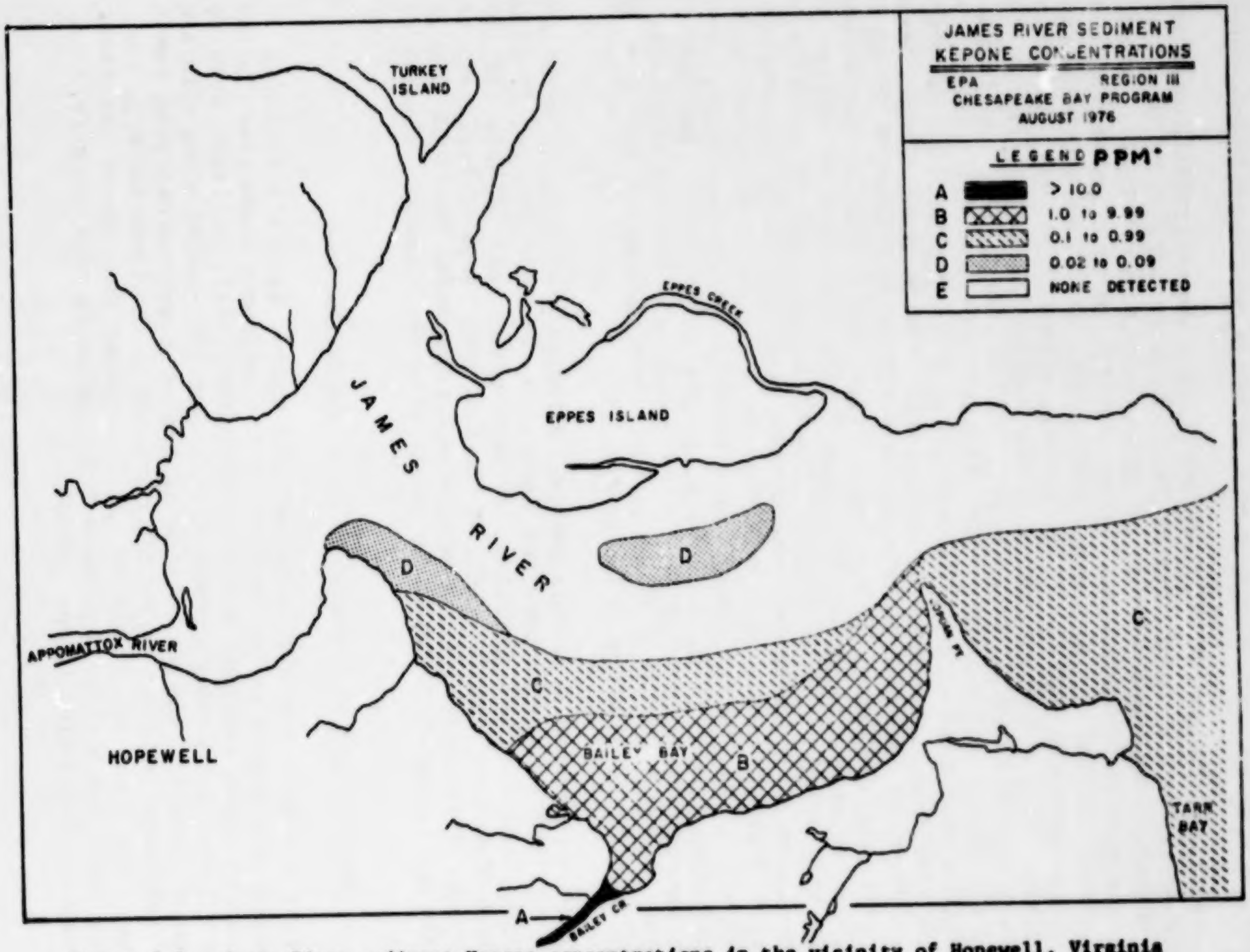
JAMES RIVER SEDIMENT
KEPONE CONCENTRATIONS
EPA REGION III
CHESAPEAKE BAY PROGRAM
AUGUST 1976
LEGEND PPM*
A ■ > 100
B ▨ 1.0 to 9.99
C ▨ 0.1 to 0.99
D ▒ 0.02 to 0.09
E ☐ NONE DETECTED
TURKEY ISLAND
EPPES CREEK
EPPES ISLAND
JAMES RIVER
D
D
C
B
C
BAILEY BAY
APPOMATTOX RIVER
HOPEWELL
TARR BAY
A
BAILEY CR

Figure 7.2. James River sediment Kepone concentrations in the vicinity of Hopewell, Virginia

Source: U.S. Environmental Protection Agency, (1976d).

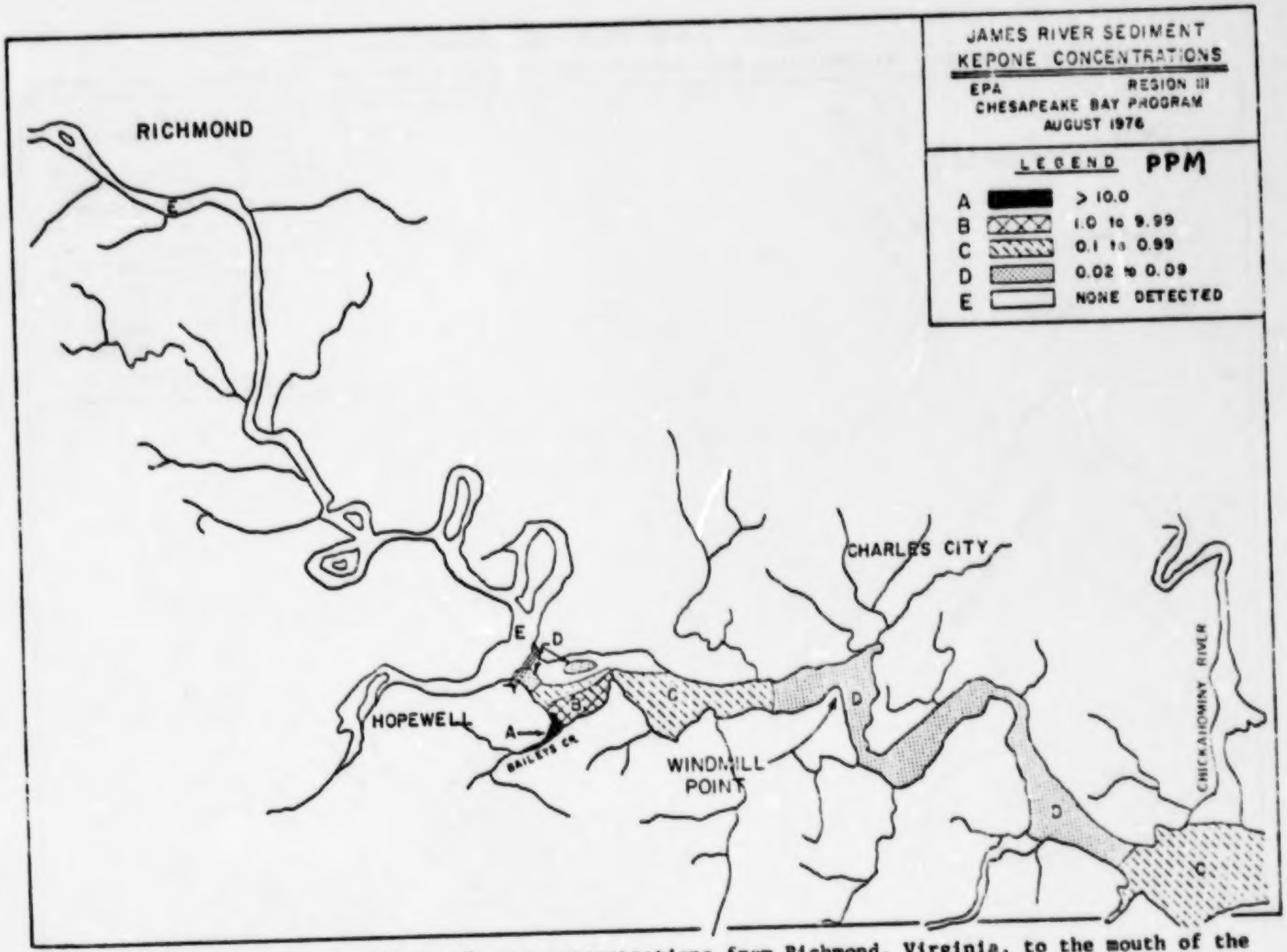
JAMES RIVER SEDIMENT
KEPONE CONCENTRATIONS
EPA
REGION III
CHESAPEAKE BAY PROGRAM
AUGUST 1976
LEGEND
PPM
A ■ > 10.0
B ▨ 1.0 to 9.99
C ▨ 0.1 to 0.99
D ▨ 0.02 to 0.09
E ☐ NONE DETECTED
RICHMOND
E
E
D
HOPEWELL
A
B
BAILEYS CR.
C
WINDMILL POINT
CHARLES CITY
D
D
C
CHICKAHOMINY RIVER

Figure 7.3. James River sediment Kepone concentrations from Richmond, Virginia, to the mouth of the Chickahominy River

Source: U.S. Environmental Protection Agency, (1976d).

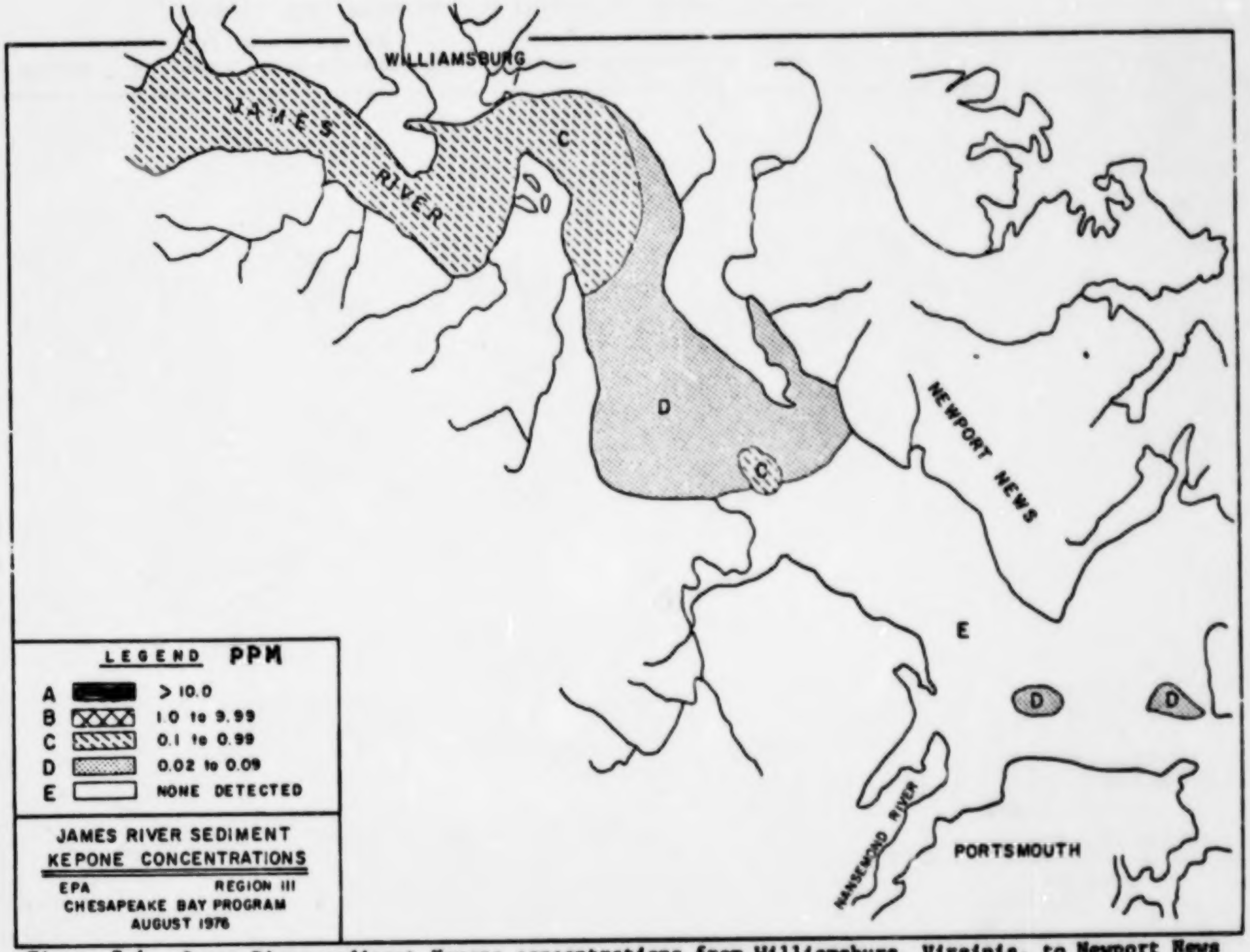
WILLIAMSBURG
JAMES RIVER
C
D
C
NEWPORT NEWS
E
D
D
NANSEMOND RIVER
PORTSMOUTH
LEGEND PPM
A > 10.0
B 1.0 to 9.99
C 0.1 to 0.99
D 0.02 to 0.09
E NONE DETECTED
JAMES RIVER SEDIMENT
KEPONE CONCENTRATIONS
EPA REGION III
CHESAPEAKE BAY PROGRAM
AUGUST 1976

Figure 7.4. James River sediment Kepone concentrations from Williamsburg, Virginia, to Newport News
Source: U.S. Environmental Protection Agency, (1976d).

7.3.4 Air

Air monitoring stations in Hopewell were located at the Hopewell News building, 200 m (0.13 mi) south of the plant site, and at the Hopewell airport 5.2 km (3.24 mi) to the east. Other sites were located in nearby communities at distances of 12.5 to 25 km (7.8 to 15.6 miles). Hi-Vol samples collected at the station 200 meters distant during the period from March, 1974 through April, 1975, while the plant was in operation, were found to contain from 2 to 50 micrograms of Kepone per m^3 of air. Kepone concentrations varied depending on the length of sampling time, weather conditions, and date of collection. During this same sampling period, the percent of Kepone in relation to the total suspended particulates (TSP) ranged from less than 1 percent to more than 40 percent.

Hi-Vol air filters taken from the Hopewell airport, the Colonial Heights area, and the most distant site (South Richmond, which is approximately 25 km (16 mi) from the manufacturing plant) contained between 0.1 and 20 ng/m^3 (U.S. Environmental Protection Agency, 1975a).

A later 1976 reentrainment investigation by EPA, with assistance from the State of Virginia, showed that some Kepone becomes airborne up to 0.8 km (0.5 mile) from the LSPC site due to local traffic and meteorological conditions (U.S. Environmental Protection Agency, 1976d).

There are no data on the mobility and persistence of Kepone in air. Air would not be a transport mode for Kepone in its insecticidal use, but it obviously was, locally at least, around the LSPC plant.

7.4 DISTRIBUTION OF MIREX AND KEPONE IN THE BIOLOGICAL ENVIRONMENT

7.4.1 Accumulation in Non-Target Organisms

Naqvi and de La Cruz (1973) investigated the quantities of mirex stored in animal tissues and thereby its transport to all trophic levels of the food web. They attempted to evaluate the incorporation of mirex in the environment on the basis of residue levels of animals collected from various habitats in Mississippi which had received varying degrees of mirex treatment Average residues of pooled samples constructed according to type of organism revealed the following hierarchy of accumulation -- annelids (0.63 ppm), crustaceans (0.44 ppm), insects (0.29 ppm), fish (0.26 ppm), and mollusks (0.15 ppm). Classification by habitat identified the highest residues among: pond (0.37 ppm), creek (0.31 ppm), grassland (0.28 ppm), lake (0.27 ppm). Lowest residues were found in the estuarine habitat (0.20 ppm). The physical parameters of the habitat were found to greatly influence the accumulation of mirex. For example, residue levels in enclosed small ponds were slightly higher than in free-flowing creeks; levels in creeks were higher than in larger bodies of lakes; and levels in lakes were higher than in the more contiguous bay-estuary. One finding of vital importance was the discovery of mirex residues in animals collected from areas with no history of direct mirex treatment, which demonstrated widespread movement of mirex in the environment. Although residue levels varied greatly, 94 percent of the samples contained less than 1 ppm mirex, and 2.5 percent of the samples contained greater than 2 ppm. Laboratory studies by Ludke, et al., (1971) in simple food chains have shown that food items containing 0.5-1.0 ppm of mirex caused up to 53 percent mortality in certain aquatic animals. The authors concluded that if the findings of Ludke, et al., can be extended to natural environments, the residue levels detected in this study are potentially poisonous to prospective consumer species which are highly sensitive to mirex.

Collins, et al., (1974) conducted a similar survey in Louisiana designed to investigate and compare accumulation of mirex residues in selected vertebrates and selected components of the human food chain for 1 year following the aerial application of mirex bait. Vertebrates in general had quite low mirex residues (0.001 to 0.005 ppm); however, certain birds such as shrikes and mockingbirds accumulated residues ranging from 1 to 8 ppm. Reptiles and amphibians generally accumulated lower residues than did birds and mammals, probably owing to the relatively greater volume of food consumed by birds on a per weight basis. As expected, predatory species of fish such as largemouth bass contained higher mirex concentrations than did omnivorous species.

Mirex was also detected in 77 percent of the human food chain items which were surveyed. These data are shown in Table 7.7. Mirex was not detected in beef fat prior to mirex treatment or 1 year after treatment. Low concentration levels were found in milk (0.001 to 0.002 ppm), chicken eggs (0.001 to 0.49 ppm), and chickens (0.004 to 0.515 ppm).

Borthwick, et al., (1973) conducted extensive field studies of coastal areas of North Carolina in order to monitor the movement and accumulation of mirex in the estuarine system following aerial application. The data revealed that (1) mirex was translocated from treated lands and high marsh to estuarine biota -- all animal classes contained mirex, and (2) biological magnification of mirex occurred -- especially in predators such as raccoons and birds. Mirex residue ranges for the respective sample categories were: water ($<$ 0.01 ppb), sediment (0 to 0.07 ppm), crabs (0 to 0.60 ppm), fishes (0 to 4.4 ppm), and birds (0 to 17.0 ppm). Mirex was not detected in any pretreatment samples of crabs, shrimp, fish, sediment, or water taken from the six monitoring stations. Table 7.8 shows the biological concentration of mirex in the estuarine food web. The authors believe that variables such as proximity to the treated area, duration of exposure to a mirex-contaminated habitat, seasonal habits, avoidance ability and position in the food web are the most important determinants of mirex accumulation levels. Additional parameters of potential importance relate primarily to the amount of mirex available in the environment including rate and method of application, amount of rainfall, surface runoff, variations in sea level, and degradation.

TABLE 7.7. MIPEX RESIDUES IN HUMAN FOOD CHAIN FROM PRETREATMENT TO 1 YEAR AFTER SINGLE MIREX APPLICATION[a,b]

Food item	Mirex Concentration, ppm													
	Pretreatment		2 weeks		1 month		3 months		6 months		9 months		12 months	
	Sample size	Residue found	Sample size	Residue found	Sample size	Residue found	Sample size	Residue found	Sample size	Residue found	Sample size	Residue found	Sample size	Residue found
Milk	1 qt	0.015	1 qt	ND[c]	1 qt	0.022	1 qt	0.001	1 qt	ND	1 qt	ND	1 qt	ND
Chicken eggs	12	ND	12	0.493	12	0.005	12	0.007	12	ND	12	0.011	12	0.001
Domestic chickens	2		2	0.004	2	0.036	2	0.515	2	0.010	2	0.201	2	0.014
Beef fat	1 lb	ND	0		0		0		0		0		1 lb	ND
Fish														
Bluegills	28	0.003	15	0.012	25	0.019	79	0.041	34	0.009	36	0.009	15	0.018
Brown bullheads	5	0.001	6	0.004	20	0.113	4	0.086	7	0.003	4	0.003	10	0.010
Largemouth bass	1	ND	1	0.018	1	0.032	1	0.624	0		0		0	
Game animals and birds														
Eastern cottontails	0		1	ND	1	ND	0		1	ND	1	0.254	0	
Opossums	1	0.120	2	0.044	0		1	0.099	1	0.004	0		0	
Bobwhite quail	1	0.113	1	0.012	1	0.475	1	1.502	0		1	0.064	2	0.036

[a] Source: Collins, et al. (1974).

[b] Average of all samples collected from entire study area at indicated sampling interval.

[c] ND=no detectable residues.

TABLE 7.8. ACCUMULATION OF MIREX IN BIOTA[a]

Sample	Residue range, ppm[b]	Posttreatment samples with mirex residues, percent
Water	<0.01 ppb	0
Sediment	0 - 0.07	3
Crabs	0 - 0.60	31
Fishes	0 - 0.82	15
Shrimps	0 - 1.3	10
Mammals	0 - 4.4	54
Birds	0 -17.0	78

[a] Source: Borthwick,et al. (1973).
[b] 0=<0.01 ppm.

The above data are illustrative of environmental concentrations resulting from the utilization of mirex as a pesticide. Some data are also available on mirex (and Kepone) accumulation in non-target organisms as a result of losses during manufacture. Mirex (or Dechlorane) has been found in fish from Lake Ontario by the Canadian Environment Ontario pesticide laboratory. Approximately 1000 fish from various locations in the Great Lakes were analyzed, but only fish from Lake Ontario were found to contain mirex residues exceeding U.S. Federal guidelines of 0.1 ppm (Anon., 1976a).

Mirex has also been found in Lake Ontario fish by the New York Department of Environmental Conservation, which has issued an order banning consumption of fish contaminated by mirex and Kepone (Anon., 1976b). Affected species are coho and chinook salmon, brown bullheads, catfish, lake trout, smallmouth bass, and members of the alewife-herring family.

Taken together, the surveys of Borthwick, et al., (1973), Naqvi and de La Cruz (1973), and Collins, et al., (1974) demonstrate quite convincingly that low levels of mirex residues remain in the environment and are concentrated by some species. Collins, et al., (1974) concluded that although all residue levels reported by their survey were well below the levels reported by others as causing acute toxicity to rats, mice, birds and fish, the lack of information on the long-range effects of low chronic dosages on non-target organisms precludes any definite statements concerning the potential long-term environmental impact of these mirex residues.

Kepone has been found in freshwater trout and suckers taken from Spring Creek, which runs near the Nease Chemical Company plant in State College, Pennsylvania. This plant produced Kepone in 1958-1959 and 1963, and manufactured mirex as late as 1974 (Anon., 1976b). Trout accumulated Kepone in amounts ranging from 0.15 to 0.17 ppm and the suckers had levels as high as 0.18 ppm (Anon., 1976a). Kepone's continued presence in this stream and its inhabitants is further evidence of its persistence in the environment. The gross losses of Kepone from the ill-equipped and poorly operated LSPC plant at Hopewell, Virginia, has caused major contamination of aquatic organisms of the James River, extending even into Chesapeake Bay (see Section 6.5.2).

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8.0 ENVIRONMENTAL ASSESSMENT

(Dr. Earl G. Alley)

8.1 ENVIRONMENTAL ASSESSMENT-MIREX

The bait technology used for this compound in control programs for the imported fire ants allows its effective utilization at very low application rates. Its use has not had a significant impact on wildlife even in estuarine areas where its impact should be the greatest. Further, its use is the only practical method for controlling an insect that poses some hazard to human health and welfare.

There is, however, the potential for problems resulting from use of this compound because it accumulates, and magnifies in food chains, is not metabolized readily, and probably causes tumors in mice and rats. Humans acquire residues, apparently as a result of use of mirex as an insecticide. Millions of acres in the Southeastern United States were formerly treated each year with low levels of this compound and it persists in the environment for many years.

The risk vs benefit of continued use of these baits is difficult to assess because neither is well defined. The risks are not proven risks and the benefits are not predominantly economic ones. The primmary environmental risk associated with continued use of mirex is to humans. They will derive some benefit from its use and in the process be subjected to certain levels of risk in terms of long term chronic effects.

Production in the United States of the toxicant for this pesticide was by Hooker Chemicals and Plastics Corporation and by several smaller companies under contract to Hooker from 1959 through 1975. Hooker did not sell any of this compound for formulation of imported fire ant baits after Allied Chemical stopped manufacture of the bait in 1975. After that time the toxicant was imported from South America and the baits manufactured by the State of Mississippi. From 1959-1975, 1,525,540 kg of this chemical were sold by Hooker. Of this quantity 400,090 kg were used for agricultural applications. In 1976-1977, 24,340 kg of technical mirex was used in the manufacture of fire ant baits (Mississippi Department of Agriculture and Commerce, 1978).

8.1.1 Uses

Use of the compound $C_{10}Cl_{12}$ as an insecticide accounted for only about 26 percent of its total utilization from 1959-1975. The portion of this production used for mirex baits within the United States was perhaps as little as 16 percent (250,000 kg). The other uses were predominantly in flame resistant polymer formulations in electronic components, television components and fabrics. The primary use of this compound as an insecticide was in a bait for the imported fire ants (Solenopsis richteri and Solenopsis invicta). This bait was called mirex and during most of the years it was employed it contained 0.3 percent toxicant. Over 100,000,000 acres were treated with 1.25 lb/ac of this formulation during a ten year period. In 1977, the formulation was changed to 0.1 percent of toxicant and the application rate to one pound per acre. About 18,000,000 lbs of this lower concentration bait was manufactured during 1977 and will be distributed until June 30,1978 (Mississippi Department of Agriculture and Commerce, 1978).

8.1.2 Analysis

Residue analysis for mirex may be done by standard methods for chlorinated hydrocarbons using extraction with suitable solvents, partitioning, Florisil cleanup, and electron capture gas chromatography. Interference from PCB's (Arochlor 1260 and 1254) may be a problem when these compounds are present. Several methods for circumventing this interference have been reported (Markin, et al., 1972; Lewis, et al., 1976). Gas chromatographic retention times provide only equivocal evidence for identity so independent confirmation is very important. Thin layer chromatography is usually too insensitive to be a useful tool for confirmation. Mass spectrometry provides a good confirmation tool. The C_5Cl_6 fragment gives intense ions at m/e 270, 274 . . . The relative intensities of these ions is determined by the ^{37}Cl : ^{35}Cl isotope ratio which provides further evidence for the elemental composition of the cluster.

8.1.3 Environmental Contamination

Contamination of the environment by mirex has been observed in the lower Great Lakes from the manufacture of the toxicant and in the Southeastern United States from its use in control programs for the imported fire ant (Collins, et al., 1974; Kendall, 1977; Spence, et al., 1974; Borthwick, et al., 1973). There is also evidence for pollution from industrial utilization of this compound (Thomas, 1978). Although large amounts of the compound have been used industrially, there are few reports of residues that can be clearly associated with this type of use.

There appear to have been two clearly established routes of mirex contamination of the environment. These are from the manufacture and industrial use of the compound and from use of the compound in programs for control of the imported fire ant. Levels in water and soil have been near or below detection limits. When watersheds treated with 0.3 percent baits were monitored mirex was found, at levels from 0.001 to 0.028 ppb, entirely associated with particulate matter. The high levels appeared only during major run-off events (Alley, unpublished results). At the current application rates, the theoretical concentration in the top three inches of soil is about 2 ppb. Concentrations found in sediments from normal bait applications have been lower than this (Spence and Markin, 1974). Sediments near manufacturing facilities either making or using mirex contained up to 1600 ppb (Thomas et al., 1978). In Lake Ontario only a few small areas are contaminated. Sediment samples from these areas average about 7 ppb (Thomas, et al., 1978). Some decomposition to dechlorinated mirex derivatives and kepone has been observed in sediments. These reactions are, however, very slow. Contamination of the biological environment by mirex has been the subject of many reports (Collins, et al., 1974; Borthwick, et al., 1973, Naqvi and de la Cruz, 1973; Kendall, et al., 1977). The contamination in the Great Lakes has resulted in residues in aquatic food chains (Hallett, et al., 1976). The residues thhat result from application of baits for fire ant control arise in both aquatic and terrestrial food chains with members of terrestrial food chains exhibiting the highest residue levels. Insectivorous birds are particularly prone to accumulate mirex residues (Collins, et al., 1974; Kendall, et al., 1977).

The only report of acute poisoning of wildlife resulting from utilization of mirex concerned crustaceans (Markin, et al., 1974). Residues in wildlife are important, however, because of potential contamination of human food supplies. Mirex is metabolized very slowly and thus is magnified in food chains and stored in the lipid part of tisses of contaminated organisms. Therefore, there exists the potential for unacceptable levels of this chemical accumulating from repeated applications over a number of years. In one study, levels in bobwhite quail from treated areas were invariably greater than established tolerances in food for human consumption (Kendall, et al., 1977).

Mirex is a very persistent chemical in the environment. Carlson, et al., (1976) found that close to half remained after twelve years. They found degradation products including hydrogen derivatives and Kepone. The sites studied by these workers were ones that had had extraordinarily heavy application of the pesticide and so these data probably reflect slower degradation rates than would be observed for normal applications. Metabolism is very slow but conversion has been reported in microorganisms (Andrade, 1975) and in monkeys (Stein, et al., 1976). Several other experiments with mammals reported that no mirex metabolism occured (Ivie, et al., 1974; Mehendale, et al., 1972; Gibson, et al., 1972; Wiener, et al., 1976).

Chemically, mirex is not very reactive. It is inert to strong acids, bases and oxidizing agents but is reactive photochemically and under a variety of reductive conditions. These reactions lead to dechlorinated derivatives of mirex and Kepone (Dilling, et al., 1967; Alley, et al., 1974a; 1974b).

8.1.4 Bioaccumulation

Uptake of mirex by plants fromm soil has been demonstrated; however, magnification does not appear to be a problem (de la Cruz and Rajanna, 1975). Bioaccumulation in aquatic species has been the subject of a number of laboratory and field studies. Hollister, et al., (1975) and Sikka, et al., (1976) have observed accumulation by algae 350 to 5000 times that in their environment. Studies with aquatic invertebrates have generally shown high bioconcentration of mirex (de la Cruz and Naqvi, 1973; Bookhout and Costlow, 1976). Field studies have verified these laboratory results (Lowe, et al., 1971). Fish accumulate mirex from their environment and from their food supply in the environment (Collins, et al., 1973) and in laboratory studies (Lee, et al., 1975; Lowe, et al., 1971). Field studies have shown accumulation of residues in the higher trophic levels, as would be expected if magnification through various food chains was occurring (Hyde, et al., 1973). Many of the laboratory experiments were performed using levels several thousand times in excess of the solubility of the compound and in some cases several million times the levels typically encountered in the environment. Therefore, these accumulation data have been obtained with unrealistic conditions and are poor models for prediction of environmental effects, particularly where solubilizing agents have been used. Neverthe- less, it is evident from field studies that bioaccumulation in aquatic ecosystems does occur.

Field studies of terrestrial species have shown that bioaccumulation of mirex is a significant problem (Mirex Advisory Committee, 1972, Hyde, et al., 1973; Collins, et al., 1974). Birds appear to be particularly vulnerable to accumulation of residues (Collins, et al., 1974; Kendall, 1977).

8.1.5 Nonhuman Toxicology

Mirex has little effect on terrestrial or aquatic microorganisms (Jones and Hodges, 1874; Brown, et al., 1975; Hollister, et al., 1975; Sikka, et al., 1976).

In contrast, invertebrate species are very sensitive to mirex. Contradictory evidence (Ludke, et al.; 1971; Muncie and Oliver, 1963) has arisen because early workers did not recognize the delayed toxic response of mirex. Not much credibility should be attached to acute studies with mirex that show low toxicity but are 96 hours or less in duration. This delayed toxicity is a major factor in the efficacy of mirex as an ant toxicant because it allows time for distribution of the compound to the entire colony including the queen and brood before toxic effects become evident. Many commercially important aquatic species are affected by low levels of this toxicant (Ludke, et al., 1971; Bookhout and Costlow, 1976; Lowe, et al., 1971). Even though mirex is very sparingly soluble in water, the acute toxic levels were generally comparable to concentrations obtainable through dissolution. Ingestion of the mirex baits (0.3 percent) by aquatic invertebrates has been shown to cause lethal toxic symptoms in these organisms (Ludke, et al., 1971; Lowe, et al., 1971). Aquatic invertebrates are the most likely organisms to suffer from pollution by mirex. Current regulations for use of the pesticide prohibit its use on aquatic habitats. The levels of mirex found in streams from areas subjected to aerial application of these baits (0.3 percent) have been 5 to 100 times less than the levels that laboratory studies have shown to be acutely toxic.

The acute toxicity of mirex to fish is very low (Van Valin, et al., 1968; Hyde, et al., 1974). Levels that did produce toxic effects were generally many thousands of times the concentration that might be expected in the environment. For example, the concentrations used by Lee, et al., (1975); Van Valin, et al., (1968); and de la Cruz and Naqvi, (1973) ranged from 1000 to 1,000,000 times realistic environmental concentrations in water.

Birds are even less acutely sensitive to mirex than fish. Dietary LC$_{50}$ values are typically in the range 1500-10,000 mg/kg. No reproductive effects have been observed with quail, mallard ducks or chickens fed 10-200 mg/kg mirex in their diet.

The acute toxicity of mirex to mammals is relatively low. The oral LD$_{50}$ values for rats are 740 mg/kg for males and 600 mg/kg for females. The acute dermal LD$_{50}$ is over 2000 mg/kg (Gaines, et al., 1969). A number of chronic effects have been observed in several mmammalian species. These include weight loss, increased liver weight, changes in liver function and structure, and reproductive problems (Gaines and Kimbrough, 1970; Khera, et al., 1976; Abston and Yarbrough, 1976; Innes, et al., 1969; Ulland, et al., 1977).

Enzyme inhibition (Hendrickson and Bowden, 1975) and changes in liver enzyme levels (Abston and Yarbrough, 1976; Baker, et al., 1972) have been observed. It is not clear, however, what significance these changes have to the organism. Increases in liver weight and fat inclusion in livers of mirex-treated animals have been observed (Abston and Yarbrough, 1976; Kendall, 1974). The distribution of mirex in tissues of rats and monkeys fed mirex contaminated diets has been investigated (Wiener, et al., 1976; Pittman, et al., 1976). Most of the residue that is retained accumulates in the fat. Significant concentrations in liver, kidney and intestine were also observed. A number of studies of the effect of mirex on reproduction in mammals have appeared. Reproductive success is impaired in rats (Gaines and Kimbrough, 1970). Placental transfer of mirex has been demonstrated (Gaines and Kimbrough, 1970; Khera, et al., 1976). Survival of offspring was reduced and cataracts developed in suckling rats from mirex contamination in the mothers milk (Gaines and Kimbrough, 1970). A reproductive study on rats by Khera, et al., (1976) showed that mirex did not produce significant changes in number of fetal skeletal anomalies at any level studied but did produce an increased number of visceral anomalies at the two highest dosages (6.0 and 12.5 mg/kg/dose; 60 and 125 mg/kg total). These levels also caused significant maternal toxicity. When male rats were treated at 1.5, 3.0 and 6.0 mg/kg/dose; (15, 30 and 60 mg/kg total) the only significant change was that animals dosed at 6.0 mg/kg/dose and mated with untreated females produced fewer pregnancies 0-5 days after dosing.

Two carcinogenicity studies with mirex have been reported. The first by Innes, et al., (1969) was with mice. This study was designed to be a preliminary screen for carcinogenicity of a large number of compounds to identify those compounds that should be subjected to more extensive studies. Of the 120 compounds tested, 11 produced significantly more tumors than controls. Mirex was one of the eleven. There was a single dosage level for each chemical and for mirex this was 10 mg/kg of body weight per day from age 7 days to age 4 weeks. After four weeks the toxicant was added directly to the diet (26 mg/kg). None of the mice on the mirex-contaminated test diet survived to the end of the experiment. Examination of these mice revealed significant increases in hepatomas in both strains of mice tested and with both males and females. Females were more susceptible than males. These preliminary data are strongly indicative that mirex caused hepatic tumors in these strains of mice. No judgments were made about whether the observed tumors were malignant or nonmalignant.

In the second study by Ulland, et al., (1973), it was initially reported that mirex was not a carcinogen in rats. Later (Ulland, et al., 1977) this interpretation of the data was changed. In this study 26 rats in each group (male and female, two dose levels) were fed 40 and 80 mg/kg for 10-weeks and then 50 and 100 mg/kg for 15.5 months. They were sacrificed at 24 months. Something apparently happened at about the 70th week of the experiment because both controls and test animals began to suffer high death rates. At the end of the experiment 35 percent of the female controls and 45 percent of the male controls had died. In the test groups survival was poorer than with the controls, falling to as low as 25 percent survival. There was a high incidence of tumors regardless of sex or dose but no statistically significant differences ($P < 0.05$) were noted with the exception of tumors of the liver. One carcinoma was detected in the low dose group, five in the high dose group (4 male, 1 female) and none in the control. The differences in this parameter between test animals and controls were not significant at $P < 0.05$. Neoplastic nodules were observed (includes the above mentioned carcinomas), six in the low dose group (2 male, 4 females), eleven in the high dose group (7 males, 4 females) and none in the control group. Only the observation of 7 neoplastic nodules in the high dose male rats was significant at the 0.05 level. This was the only statistically significant result from the data of this study that indicates that mirex produces tumors. Although the experimental design and circumstances did not allow statistically valid conclusions to be drawn from most of these data, the apparent trend of higher rates of liver alteration, tumors and carcinomas makes it clear that well-designed studies are needed. A good summary of the state of knowledge with regard to the carcinogenic potential of mirex would be that mirex is possibly cancer causing, but has been neither established as carcinogenic nor proven safe.

8.1.6 Effects on Human Health

There has been no exposure of humans to mirex equivalent to the Kepone exposure of Life Sciences Products Company employees. Therefore, no data are available on the toxic effects of mirex to humans. Monitoring data indicate that humans are exposed to and are acquiring residues of this compound. The highest level reported was 1.32 ppm in adipose tissue (Kutz and Strassman, 1976). Acute toxic effects would not be expected to be readily apparent from contamination at these levels. Only about ten years have elapsed since aerial applications were begun and so cancer epidemiology studies would not yet be expected to reveal if mirex is a carcinogen in humans. The exposure of human beings to this compound is not very great and so no group of individuals with significant exposure to the chemical is available. Some judgments about the effects of this compound on human health can be drawn from other studies. The potential for problems clearly exists because mirex is not metabolized by mammals (Ivie, et al., 1974); it is stored in the fat (Mehendale, et al., 1972) and is excreted very slowly (Ivie, et al., 1974), therefore, prolonged exposure even at very low levels could lead to significant body burdens of the chemical. Residues have been observed in human adipose tissue and extrapolation of the data of Ivie, et al., (1974) would lead to an intake of about 0.01 mg/kg to give the equivalent amount of toxicant in a rat. The studies showing chronic toxic effects have utilized levels from 2500 to 10,000 times this concentration. These studies are indicative that given sufficient exposure effects on human health may occur. This risk must be weighed against the human health problems posed by the imported fire ant. Presently mirex is the only practical toxicant for wide area treatment of this insect.

8.1.7 Regulations

The established tolerances for mirex are 0.1 ppm (negligible residue) in the fat of meat of cattle, goats, hogs, horses, poultry and sheep; 0.1 ppm (negligible residue) in milk fats and eggs; and 0.01 ppm (negligible residue) in or on all other raw agricultural commodities (Code of Federal Regulations, 1976). These regulations appear to provide adequate protection of human food supplies.

8.2 ENVIRONMENTAL ASSESSMENT-KEPONE

The registered uses of Kepone do not appear to present a significant hazard to the environment. That careful control of manufacturing operations for toxic chemicals is necesssary was painfully illustrated by the problem created by Life Science Products Company's misuse of this chemical. It is difficult to make objective judgements about this compound because of the highly emotional atmosphere that surrounds it. However, even if it had not been misused by LSPC, the fact remains that it has been identified as a carcinogen in rats and mice. Human exposure has been very limited and so the benefits of its household use as an ant and roach bait must be weighed against the potential accumulation and the possibility that this could cause cancer in humans. Continued use of Kepone does not appear to present an environmental hazard if its use is controlled very carefully.

Residues present in the environment and in human food supplies are a pressing problem. Ways of decontaminating sediments need to be explored and careful attention paid to residues in food from these contaminated areas.

Kepone was produced by Allied Chemical Corporation and various contractors from 1951-1975. During this period 1,592,895 kg were produced (Ferguson, 1975). Almost all of this quantity was manufactured for export (U.S. Environmental Protection Agency, 1976). Over half of the total quantity was produced from 1974 through July 1975 (Ferguson, 1975) when production was terminated. Kepone is no longer being manufactured in the United States.

8.2.1 Uses for Kepone

The primary uses for Kepone were as a chemical intermediate for production of an insecticide (Kelevan) in Europe and for direct use in control of the banana root borer. In the United States the registered uses have been for wireworm control in tobacco and in 0.125 percent bait formulations for household control of ants and roaches.

8.2.2 Analysis

The analytical methods for Kepone are similar to the standard chlorinated hydrocarbon screening procedures (Pesticides Analytical Manual). Kepone adsorbs strongly to glass and other surfaces. This can lead to contamination problems (syringes, glassware, etc.) or to losses. Kepone readily forms a hemiketal with alcohols and so if one introduces methanol or ethanol into the solutions less problem with adsorption is encountered and the gas chromatographic behavior is not changed, because reversion to Kepone occurs in the heated inlet of the gas chromatograph.

Florisil cleanup is not as satisfactory as with less polar compounds because the more polar eluates also elute many polar interfering substances. Advantage may be taken of the solubility of Kepone in aqueous alkali solutions to help remove interferences.

Confirmation by mass spectrometry can be achieved. The ions m/e 270, 272, 274 . . . for C_5Cl_6 are very intense and their relative intensities must be in accord with the expected isotopic abundance of chlorine. A weaker cluster for C_5Cl_4O is also observed.

8.2.3 Environmental Contamination

The only significant source of environmental pollution by Kepone in the United States has been from facilities producing the technical grade chemical.

Contamination of the environment does not appear to be a problem when Kepone is used as prescribed, because the uses authorized for this compound in the U.S. utilize it in very low concentration in baits and traps with no broadcast application. The major environmental problems that Kepone poses are associated with the manufacture of the technical material.

The situation that arose at LSPC was hopefully a unique one. Unauthorized dumping and poor containment of dust and powders in the manufacture of the compound resulted in air and water pollution of an unprecedented magnitude. It is unlikely that this will be a recurring problem but the massive contamination that did occur is likely to remain as a problem for years because of the environmentally stable nature of the compound.

Contamination of wildlife, in particular aquatic organisms, appears in all cases to be associated with manufacture of the pesticide, never with its normal utilization as a commercial poison. This contamination must be of particular concern when the organisms are sources of human food.

Kepone appears to be a very persistent pollutant. Residues have been detected in biota in contaminated ecosystems more than ten years after the last exposure (Toxic Materials News, 1976). However, Kepone is chemically reactive. The facile condensation and nucleophilic displacement involve the gem dihydroxy group, not the chlorinated carbon skeleton. Kepone may be regenerated by degradation of the compounds that result from these reactions (Sandrock, et al., 1974). At short wavelengths, Kepone is more photochemically reactive than mirex (Alley, et al., 1976) and its photochemistry involves reductive dechlorination (Alley, et al., 1974a)

8.2.4 Bioaccumulation

Aquatic invertebrate species accumulate Kepone efficiently from water containing this pollutant by factors ranging from 10 to 10,000 (Bahner, et al., 1976; Hansen, et al., 1976). Aquatic vertebrates also were found to concentrate Kepone from contaminated water (Bahner, et al., 1976). Both invertebrate and vertebrate species accumulate Kepone from contaminated food sources (Bahner, et al., 1976). No data on metabolism have been reported.

Accumulation of Kepone residues in mice and rats was observed when this compound was included in their diets. These residues declined sharply if the animals were removed from the contaminated diet (Huber, 1965). Dairy cattle fed Kepone-contaminated rations produced milk contaminated with Kepone residues. These residues dropped quickly when a normal diet was resumed (Smith and Arant, 1967).

It appears that Kepone behaves similarly to the other chlorinated hydrdocarbons in terms of uptake from a contaminated environment. It does appear to be much more readily excreted than many of the other members of this group (Huber, 1965). This is especially evident when Kepone is compared to mirex. It appears that incorporation of residues can result both from environmental exposure (air and water) and from contaminated food sources.

8.2.5 Nonhuman Toxicology

Laboratory experiments have shown that Kepone is a toxic to microorganisms (Brown, et al., 1975) and levels from 0.35 to 0.60 ppm caused a 50 percent reduction in growth of phytoplankton (Walsh, et al., 1976). Under controlled laboratory conditions, Kepone is very toxic to some aquatic invertebrates - mysid, 96 hr LC_{50} 10.1 ppb (Nimmo, et al., 1976) but less so to some others - blue crab, 96 hr LC_{50} 1345 ppb (Heitmuller, 1975). Toxicity of Kepone to aquatic vertebrates is in this same range, 48 hr LC_{50} 50-100 ppb, (U.S. Environmental Protection Agency, 1976). Concentrations found in contaminated waters have all been under 4 ppb, less than the lowest of these LC_{50} values. The higher level in sediments (up to 30 ppm) from contaminated areas is probably more significant to the inhabitants of these aquatic ecosystems. No laboratory studies addressing the question of the effect of contaminated sediments on these organisms are available. Kepone has not been a pollution problem outside the aquatic environment except where terrestial species are at the upper end of aquatic food chains. Birds and mammals are candidates for this type of exposure. The acute toxicity of Kepone to several species of birds is low; however, chronic efffects have been noted (Eroshenko and Wilson, 1974). These included liver enlargement, weight loss, tumors, changes in the reproductive organs and an "estrogen-like" effect of the compound. It is likely that carnivorus birds in the contaminated area will achieve significant residues of Kepone, but it is unlikely that this exposure will approach the levels used in the laboratory experiments (50-600 ppm feeding levels).

While the effects of Kepone on mammals are of interest from the standpoint of survival of wildlife, their primary importance lies in their relationship to human health. Kepone presents an unusual case. In addition to the usual experiments with laboratory animals there is also a human population that has been subjected to high exposure.The human subjects exhibited symptoms similar to those produced in test animals subjected to sublethal doses, notably neurological symptoms, tremors, irritability, weight loss and reproductive failure (Center for Disease Control, 1976).

Acute toxicity values of Kepone for several mammalian species have been determined and are in the range 70-250 mg/kg. Thus, Kepone has moderate acute toxicity compared to other poisons. Chronic toxicity studies have shown, in addition to those effects listed above, rapid accumulation of residues followed by relatively rapid loss of residues after withdrawal from the contaminated diet; reproductive problems-loss of both male and female fertility, placental transfer, contamination of milk; teratogenic effects, and increased incidence of oncogenicity and carcinogenicity in mice and rats (U.S. Dept. HEW, NIH-NCI, 1976). When compared to pooled controls both male and female rats at the high dose rate showed statistically significant (P 0.05) increases in hepatocellular carcinomas.

Male and female mice showed significant increases at both high and low dose rates. It is clear from this study that Kepone produced cancer in both rats and mice fed diets contaminated with 8 to 40 mg/kg of toxicant.

8.2.6 Effects on Human Health

The information available on toxicity of Kepone to humans results from the Life Sciences Products Company workers who were exposed to sublethal doses of this chemical. The exposure occurred from 1974 through 1975 and so no information on long term effects such as carcinogenicity, mutagenicity or teratogenicity can be derived from studies of these individuals. Some of these people were heavily exposed and suffered sublethal toxic effects. These included neurological impairment manifested by tremors, jumpy eye movements, exaggerated startle response and cerebellar dysfunction (Guzelian, et al., 1975); hepatotoxic effects-histology showed increased fat, numerous dense bodies and proliferation of smooth endoplasmic reticlum. These symptoms are not unexpected from individuals suffering heavy exposure to organochlorine pesticides. In addition, certain reproductive effects were observed including decreased fertility manifested by low sperm counts, decreased motility and increased abnormal forms (Anderson, et al., 1976).

It is unlikely that members of the general public would have sufficient exposure to suffer from acute Kepone poisoning. Chronic poisoning from contaminated food could be a problem if careful attention is not concentrated on residues in food taken from contaminated waters.

8.2.7 Regulations

The Food and Drug Administration has established action levels for residues of Kepone in fishery products as follows: oysters, clams and mussels 0.3 ppm; fin fish 0.1 ppm; crabs 0.4 ppm, based on edible portion in each case. These "action levels" are based on average dietary intake of the mentioned foods, and appear to provide adequate protection.

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